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A COMPARISON OF THE METABOLISM OF MOSAIC DISEASED POTATOES WITH THAT OF NORMAL POTATOES

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Scottish Plant Breeding Station, Edinburgh

(With 8 Text-figures)

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I. INTRODUCTION

THE effects of mosaic diseases upon carbohydrate and nitrogen relations of host plants have received considerable attention. A wide range of plant species affected with many types of mosaic disease have been studied and certain general conclusions may be drawn from the observations recorded in the literature. Most workers are agreed that in actively growing plants mosaic diseases are accompanied by disturbances in carbohydrate metabolism which are revealed in reduced amounts of carbohydrates, particularly of polysaccharides. Starch/soluble-sugar relationships have been found to be chiefly affected and degradation of starch to be retarded. Observations on nitrogen content have shown that nitrogen is usually higher in diseased plants and degradation of nitrogenous materials is affected in similar fashion to degradation of

carbohydrate, i.e. insoluble forms are less readily hydrolysed. In all cases there has been indication of a lower C/N ratio in diseased plants as compared with healthy plants.

The present investigation has confirmed and extended some of these observations. It has also provided results which controvert, in part, and extend those previously published upon the nature of carbohydrate metabolism in normal potato leaves (Davis & Sawyer, 1916; Clements, 1930; Barton Wright & McBain, 1932, 1933*a*), and it is most convenient, therefore, to present and examine first those data obtained from the healthy material. The findings of this examination will serve, subsequently, as a basis upon which to establish a comparison between the metabolism of mosaic affected leaves and that of normal leaves.

This work formed part of an investigation designed to examine comprehensively, in relation to the continuity of metabolic change, the carbohydrate, nitrogen and mineral metabolism of normal and mosaic diseased potato plants. The investigation had to be curtailed in 1934 before reaching completion, and only those data relative to carbohydrates and total nitrogen in leaves and petioles are available for discussion.

II. Material and methods

The potato varieties used throughout the investigation were President and Arran Victory. The material for each variety consisted of sixty diseased plants, infected with potato virus *X* and showing symptoms of mild or simple mosaic, and sixty proved virus-free plants to serve as healthy controls. All the plants were grown in a single insect-proof greenhouse, and comparable conditions were maintained between individuals by equality of treatment and frequent change of position. Random samples of twenty whole leaves from the intermediate regions of the stem were taken at intervals of 2 hr. over each of the diurnal periods to be considered. In each case, except that of 20 days old President, the leaves were fully expanded at the time of sampling.

Estimates of the seasonal variations in total carbohydrates and total nitrogen were obtained from samples of twenty leaves removed at weekly intervals from the plants used in diurnal experiments. The weekly samples from Arran Victory were obtained at 10.0 a.m. G.M.T. and those from President at 11.0 a.m. G.M.T.

The treatment of samples in preparation for analysis was similar to that used by Barton Wright & McBain (1933*a*, *b*), and drying was carried out in two stages as recommended by Link & Tottingham (1923). This

method has been shown to be satisfactory for both carbohydrates and nitrogen (Pearsall & Wright, 1929).

All carbohydrate analyses were effected by the Schaffer & Hartmann (1921) microtechnique, and total nitrogen was estimated by the method described by Ranker (1925) which includes nitrate-nitrogen in the estimate.

Calculations for diurnal material are based upon the residual dry weight (R.D.W.) (Mason & Maskell, 1928), and the standard error is attached to each calculated mean value. In order to express more clearly the seasonal differences in carbohydrate relationships, the data for each diurnal sample have been calculated also as percentages of the total estimated carbohydrates and subjected to the same treatment as that given to the actual values. Comparisons between mean values have been examined for significance by a calculation of the ratio between difference of the mean and error of the difference, shown in the tables as D/E_D. The odds against D/E_D exceeding 2·80 by accident in a comparison of 24 values are 100 : 1 (see Table of t, Fisher, 1932), so that any value of this ratio greater than 2·80 may be considered indicative of a fully significant difference between the two mean values compared.

III. THE CARBOHYDRATE METABOLISM OF THE NORMAL LEAF

Experimental observations

Diurnal series.

President, 20 days old. The mean values about which diurnal fluctuations of reducing sugars, sucrose and starch occurred in the leaves and petioles of 20 days old President are given in Table I. These data show that reducing sugars predominated in the leaf blade whilst sucrose predominated in the petiole, a result which is contrary to those previously recorded for the potato (Davis & Sawyer, 1916; Clements, 1930; Barton Wright & McBain, 1932) and to the later observations on the present material. Similar high values of reducing sugars were found by Went (1898), however, in those parts of the sugar cane in which rapid extension growth was taking place. The potato leaves sampled at 20 days were not

Table I

	Lamina		Petiole
Mean values	% R.D.W.	% total carbohydrate	% R.D.W.
Reducing sugars	0·973 ±0·040	44·20 ±1·46	0·274 ±0·076
Sucrose	0·376 ±0·035	16·95 ±1·36	1·183 ±0·050
Starch	0·854 ±0·022	38·91 ±0·36	0·368 ±0·060

fully expanded, and there is evidence to suggest that leaves on plants of this age would be developing rapidly through cell differentiation (Stone, 1933) and increasing in weight (Bunzel, 1914). It is not unreasonable, therefore, to interpret the data in Table I as indicative of a metabolism conditioned to support growth activities.

A high reducing sugar content in growing regions was suggested by Went to be linked with the utilization of carbohydrate in growth requirements, since reducing sugars may serve both as substrates in the synthesis of complex carbohydrates and also as the most suitable sugars for the establishment of the high internal pressures necessary to cell extension. Onslow (1931) has accepted and extended these arguments on biochemical

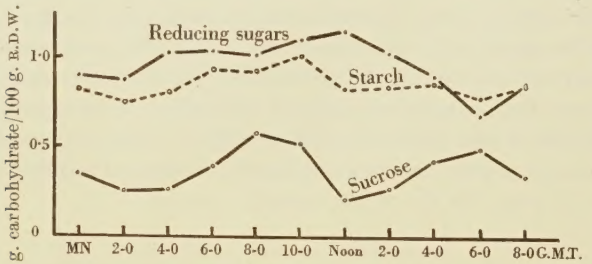

Fig. 1. Diurnal variations in carbohydrates of expanding leaves of healthy President at 20 days.

grounds. Priestley (1929), in a study of cell differentiation, has also stressed the high carbohydrate requirements of growing tissue in the formation of permanent structures, e.g. cell walls. It would seem, therefore, that in the young expanding potato leaf diurnal fluctuations in "free" carbohydrate would be reflexions of the disturbances in balance between utilization in support of growth and supply through carbon assimilation. Examination of the curves in Fig. 1 confirms this view. The accumulation of each form of carbohydrate with increasing light intensity between 4.0 a.m. and noon may be interpreted as the formation of sugars at a rate greater than that of utilization. From the order in which the curves begin their upward trend it would seem that reducing sugars accumulated first and that condensation to starch and sucrose was, subsequently, a concomitant of this accumulation. After noon, and with decreasing light intensity, the diminished values of reducing sugars may be taken to indicate a diminished rate of formation. When the concentration of reducing sugars reached a minimum value at 6.0 p.m. there is indication that reinforcement from sucrose and starch took place

and that these latter ultimately formed a source of supply of carbohydrate within the leaf.

Upon this interpretation, sucrose and starch serve as temporary storage products for excess carbohydrate formed in photosynthesis (cf. Clements, 1930; Barton Wright & McBain, 1932), and the balance of metabolism is controlled by the requirements of the leaf as a growing organ. Photosynthetic activities are conditioned to supply the materials of these requirements and, in consequence, the levels of sucrose and

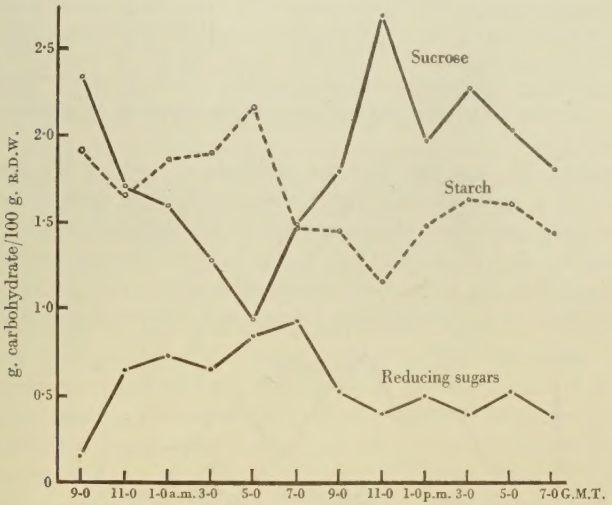

Fig. 2. Diurnal variations in carbohydrates of the leaves of healthy President at 44 days.

starch are low in relation to that of reducing sugars upon which, presumably, considerable demands are made in support of respiratory and synthetic activities. On the other hand, when conditions are favourable for the maintenance of carbon assimilation, that is during daylight, photosynthetic activity is sufficiently strong to throw the balance temporarily in favour of carbohydrate formation and the accumulation of reserve products.

President, 44 days old. The diurnal fluctuations and mean levels of carbohydrate in the leaves and petioles of 44 days old President (Fig. 2, Table II) are in close agreement with those previously recorded for potatoes in a vigorous vegetative condition prior to flowering (Davis & Sawyer, 1916; Clements, 1930; Barton Wright & McBain, 1932). The most

probable interpretation of these data is that carbohydrate formation during the day takes place in excess of utilization with the result that sucrose and starch accumulate as temporary reserve products. The progressively decreasing amounts of sucrose and starch during the night,

Table II

	Lamina		Petiole
Mean values	% R.D.W.	% total carbohydrate	% R.D.W.
Reducing sugars	0·564 ± 0·061	14·42 ± 1·64	0·888
Sucrose	1·780 ± 0·152	44·21 ± 3·15	0·936
Starch	1·636 ± 0·072	41·36 ± 2·02	1·655

accompanied by increase in reducing sugars, points to the hydrolysis of starch to hexoses and the removal of soluble sugars in synthesis and/or by transport.

President, 86 days old. The carbohydrate curves for the leaves of President in a post-flowering and pre-senescent stage present a totally different appearance from those provided by the material sampled earlier in the season. It will be seen (Fig. 3) that between 9.0 a.m. and 1.0 p.m. the curves follow the normal daytime trend suggestive of photosynthetic activity resulting in carbohydrate formation in excess of utilization and

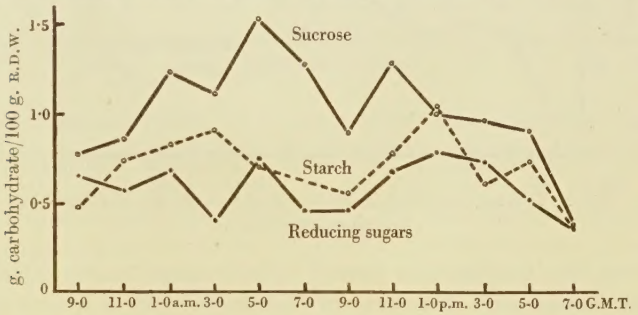

Fig. 3. Diurnal variations in carbohydrates of maturing leaves of healthy President at 86 days.

transport. The downward trend of all three curves between 1.0 p.m. and 7.0 p.m. is also normal and readily interpreted as showing a reduction of synthetic activity with decreasing light intensity and a consequential removal of the temporary storage products. Between 9.0 p.m. and 5.0 a.m., however, there was a marked increase in all forms of carbohydrate but chiefly in sucrose and starch.

Nocturnal increases in carbohydrate have been observed previously in leaves of potatoes approaching senescence (Clements, 1930; Barton

Wright & McBain, 1933 *a*) and also in mangold leaves sampled in September and October (Davis *et al.* 1916). It would appear, therefore, that the phenomenon is intimately associated with the onset of maturity and/or the transfer of carbohydrate reserves from leaves to winter storage organs. There is abundant evidence that annual plants at the end of their period of growth decrease considerably in dry weight (Blackman, 1919; Miller, 1931), and, although such a decrease cannot be substantiated in fact by the present data, the course of events shown (Fig. 3) would appear to indicate that during the pre-senescent period in the potato, carbohydrate reserves of the leaves are hydrolysed and removed to the storage organs. It would seem, indeed, that hydrolysis of the more permanent reserves, e.g. hemicelluloses, glucosides, and pentosans (Davis & Sawyer, 1916; Clements, 1930), may take place to such an extent during the night that there is a necessary recondensation of the simple sugars formed in hydrolysis to temporary storage products, in which form they may be held readily available for transport.

There are difficulties which arise in accepting this explanation of the nocturnal increase in carbohydrates, particularly with regard to the enhanced values of sucrose. The formation of sucrose is dependent upon supply of glucose and γ-fructose, and whilst there is strong direct evidence that glucose is the main product in the hydrolysis of polysaccharides and carbohydrate derivatives in general there is little proof that γ-fructose may arise in this way. Onslow (1931), however, has assembled evidence to show that starch and sucrose are interconvertible. The mechanism suggested is based upon the assumption that active, unstable γ-hexoses are formed in hydrolysis, and that in this active condition γ-glucose and γ-fructose are interconvertible. If this hypothesis be accepted and applied to the circumstances obtaining in the material under discussion, it would seem that the products of hydrolysis of reserve substances in the leaf are determined by two factors, first, the equilibria of the soluble sugars and, secondly, the rate of transport of these latter from the leaf. In other words, the conditions which determine the forms in which carbohydrate accumulates during the night when the primary forms of sugar are derived by hydrolysis are similar to those obtaining during the day when the primary sugars arise *de novo*. It has already been indicated that during the daylight period the normal synthesis of carbohydrate takes place, and it is suggested, therefore, that the hydrolysis of reserve products is controlled by the level of soluble carbohydrates, particularly of the unstable forms, within the leaf. Thus when photosynthetic activity is resumed in the early morning with the consequent

production of reducing sugars, the conditions become unfavourable for hydrolysis which, in consequence, ceases. At night, on the other hand, photosynthesis is no longer active and the conditions favour hydrolysis.

From the above discussion it would appear that metabolic activities in the maturing potato leaf are directed chiefly towards the supply of soluble sugars for transport and storage in the appropriate organs, namely, the tubers. A comparison to be made later of the petiolar data for each of the diurnal periods will corroborate this view.

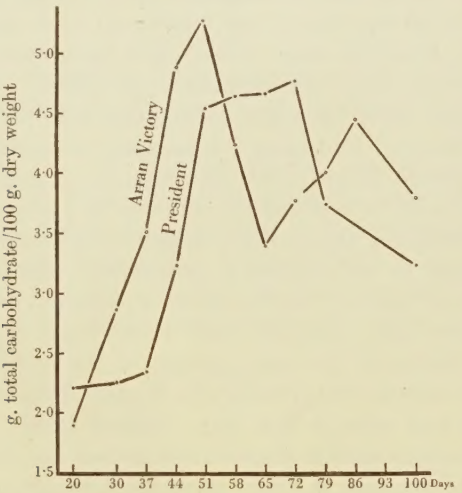

Fig. 4. Seasonal variations of total carbohydrate in healthy leaves of President and Arran Victory.

Seasonal series.

President. Seasonal variations in total weak-acid-hydrolysable carbohydrates of President leaves (Fig. 4) are expressive of three major periods of leaf metabolism. During the first period, 20–37 days, carbohydrate values remained at low levels although there was a slight tendency towards increase. Later this tendency became marked and the second period was characterized by a rapid increase in values culminating in a maximum between 51 and 72 days. Finally, there was a progressive diminution of carbohydrate from 72 days onwards. These data corroborate the interpretation placed upon the results of diurnal investigation, since they indicate that in expanding leaves utilization of carbohydrate is almost complete; that in fully expanded leaves carbohydrate is formed in

excess of utilization; and that in maturing leaves there is a withdrawal of carbohydrate reserves.

Arran Victory. The Arran Victory plants used in this investigation grew so strongly during the early part of the season that they rapidly became pot-bound and showed signs of premature ripening. In order to check this tendency each plant was repotted and supplied with a single teaspoonful of mixed fertilizer immediately after the seasonal samples were obtained on the 58th day. In response to this treatment a new period of growth was initiated. The effect of ripening and of treatment is seen plainly in the curve for total carbohydrates (Fig. 4).

The first four values on the curve were obtained from samples taken before the ripening effect was visible. During this period there was a rapid increase in carbohydrate content culminating in a maximum at 51 days. The premature ripening effect was apparently responsible for the rapid decrease in carbohydrate values between 51 and 58 days, but after treatment on the latter date, and presumably in response to the more favourable conditions, the decrease was checked at 65 days. Thereafter the curve shows an increase in carbohydrate to 86 days and a decrease from 86 days onwards. With the exception of the effect traceable to abnormal conditions of growth, the curve is very similar to that for President. This similarity is shown also in the seasonal distribution of the individual carbohydrates (Table III).

Table III

	% R.D.W.			% total carbohydrate		
Mean values	20 days	30 days	72 days	20 days	30 days	72 days
Reducing sugars	0·720	0·407	0·591	36·55	15·06	22·04
Sucrose	0·768	1·416	1·028	38·98	53·92	37·64
Starch	0·463	0·803	1·035	24·47	31·02	40·35

The major points of difference between the data for Arran Victory and for President are those of time and actual amounts of carbohydrate. Thus, Arran Victory appears to pass through its early stages of growth rapidly and, under normal conditions, might be expected to show maturity changes in metabolism earlier. In general, however, seasonal changes in the metabolism of carbohydrates in Arran Victory appear to follow the same lines as those adduced for President.

Petioles.

The estimates of carbohydrate variations in the petioles of both President and Arran Victory have been restricted to group analyses of

starch, sucrose and reducing sugars for each of the diurnal periods investigated. The mean diurnal values for each variety are given in Table IV and arranged in progressive series with age, irrespective of variety.

Table IV

	% R.D.W.				
Mean values	President 20 days	A. Victory 30 days	President 44 days	A. Victory 72 days	President 86 days
Starch	0·368	1·581	1·655	1·702	1·086
Reducing sugars	0·274	0·694	0·888	0·970	2·689
Sucrose	1·185	0·687	0·936	1·054	1·060
Soluble sugars	1·457	1·381	1·824	2·024	3·749

It will be seen from Table IV that soluble sugars in the petioles increased progressively throughout the season and reached very high values in the 86 days old material. Over the whole period, however, sucrose values remained almost constant, and the variations in soluble sugars were due entirely to increases in reducing sugars. These facts are in complete accord with those of other investigators, and the most probable interpretation of them is that they indicate a progressive increase in the amount of carbohydrate transported from the leaf. The increase is particularly well marked in the 86 days old plants and is corroborative, therefore, of the trend of metabolism suggested for these plants, namely, that depletion of carbohydrate in the leaf is directed towards transport of reserve materials from the leaf to the tubers.

The nature of the sugars concerned in translocation in the potato has given rise to two contrary views. Davis & Sawyer (1916) and Clements (1930) have expressed the opinion that since reducing sugars preponderate in the petioles they must be the chief sugars of transport. On the other hand, Barton Wright & McBain (1932) claim that sucrose is the translocatory sugar. Their data are conclusive in establishing an obligate connexion between sucrose and the phloem of the conducting organs and also in showing sucrose to be *a* form of sugar in which carbohydrate is translocated in the healthy potato plant. The data do not preclude, however, the possibility that reducing sugars are also concerned in transport. On the contrary, the results from the leaf-roll material would appear strongly to favour reducing sugars as the more readily translocated, since a positive gradient of hexoses between the midribs of the leaves and the lower stems was traced in these plants.

The present evidence, although inconclusive with regard to the function of sucrose in the petiole, supports the contention that reducing

sugars are the chief forms in which sugar is translocated, particularly towards the end of the plant's life, since reducing sugars in the petioles increased progressively in amount during the course of the season whilst sucrose remained almost constant. This latter fact may indicate that translocation of sucrose takes place at a maximum rate throughout the season and that it is restricted to certain channels, namely, the phloem. If this be the case, then it would seem that translocation of reducing sugars suffers no restrictions of this nature and has alternative channels as was suggested for the leaf-roll material of Barton Wright & McBain (1932).

IV. VARIATIONS IN THE CARBOHYDRATE AND TOTAL NITROGEN CONTENT OF MOSAIC DISEASED LEAVES AS COMPARED WITH NORMAL LEAVES

Before proceeding to a detailed comparison of the diurnal and seasonal variations in healthy and mosaic-infected plants, it is convenient to draw attention to a salient feature of the results to be presented. It will be observed from the data for each series of comparisons that the *gross* levels of the carbohydrates with regard to seasonal changes in level, and the *gross* trend of diurnal and seasonal variation are closely similar in both healthy and diseased material. These facts of similarity may be used in dual fashion: first, to double the strength of the evidence relative to metabolism in the healthy plant; and secondly, to establish the principle that the *gross* metabolism of the diseased plant is essentially that of the healthy plant. An acceptance of this principle at this stage will avoid constant repetition in drawing attention to the fact that the differences to be found in detailed comparison are small in relation to the similarities in wide comparison.

Experimental observations

Diurnal series.

President, 20 days old. The slight tendencies towards difference between the curves and mean values for mosaic-diseased and healthy leaves of 20 days old President (Fig. 5, Table V) are worthy of examination in view of their bearing on the further results. The curves show that reducing sugars in the diseased laminae tended to fluctuate at a lower mean level during the day and at a higher mean level during the night. The diurnal mean (Table V), however, was slightly lower than that for the healthy laminae. Sucrose values, on the other hand, were almost identical for both series of plants at all times of day and also in the diurnal means.

Starch fluctuations resembled those of reducing sugars, in that they took place about a lower mean value in the diseased laminae, and it will be observed that they showed a tendency to lag behind corresponding fluctuations in the healthy laminae. When considered in proportionate relationships, the mean values for reducing sugars were almost identical

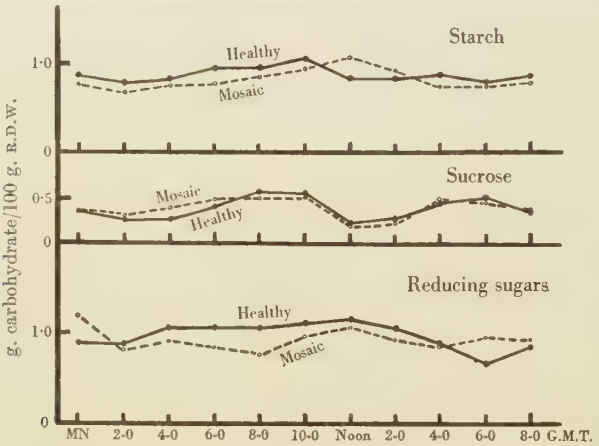

Fig. 5. Diurnal variations in carbohydrates of expanding leaves of healthy and mosaic President at 20 days.

Table V

Mean values	Healthy	Mosaic	Difference	D/E_D
% R.D.W.:				
Reducing sugars	0·973 ±0·040	0·939 ±0·036	0·034 ±0·054	0·63
Sucrose	0·376 ±0·035	0·382 ±0·034	0·006 ±0·049	0·12
Starch	0·854 ±0·022	0·803 ±0·034	0·051 ±0·041	1·24
% Total:				
Reducing sugars	44·20 ±1·46	44·24 ±1·23	0·04 ±1·91	0·02
Sucrose	16·95 ±1·36	17·99 ±1·47	1·04 ±2·01	0·52
Starch	38·91 ±0·36	37·77 ±1·16	1·14 ±1·21	0·94

in both series of plants, and the balance of difference was held by slightly higher sucrose and slightly lower starch in the diseased leaves.

The main direction of metabolism in the leaves of normal potatoes at this stage of development has been postulated to be that of carbohydrate formation and utilization in tissue synthesis. The facts for the diseased laminae give support to this hypothesis and, therefore, the slight differences which have been noted must be ascribed to either an excess of utilization or a diminished rate of formation of carbohydrate in these

laminae. The fact that reducing sugars and starch were most markedly lower during the earlier part of the photosynthetic period is suggestive of a lower rate of formation as most probable. Since the higher values of reducing sugars in the diseased laminae during the night may be taken to indicate that the demand made upon these sugars in support of respiration and synthesis was not as great as in the healthy laminae, then the argument in favour of a lower rate of carbohydrate formation is considerably strengthened. The lag in starch fluctuations together with lower starch values on both actual and proportional bases lead to the further suggestion that starch formation is subject to additional impediment in the diseased laminae.

Table VI

Mean values	Healthy	Mosaic	Difference	D/E_D
% R.D.W.:				
Reducing sugars	0·564 ±0·061	0·464 ±0·066	0·100 ±0·090	1·11
Sucrose	1·780 ±0·152	1·601 ±0·166	0·179 ±0·225	0·80
Starch	1·636 ±0·072	1·434 ±0·081	0·202 ±0·108	1·87
% Total:				
Reducing sugars	14·42 ±1·64	13·63 ±2·05	0·79 ±2·63	0·30
Sucrose	44·21 ±3·15	44·71 ±3·11	0·50 ±4·43	0·11
Starch	41·36 ±2·02	41·66 ±2·78	0·30 ±3·44	0·10

President, 44 *days old*. If the effect of virus infection is to decrease the efficiency of carbohydrate production, as indicated by the data for the young developing leaves, then stronger evidence of this diminished efficiency should be obtained when the trend of carbohydrate metabolism in the leaf is directed towards the formation of storage carbohydrates. The comparative data in Table VI show that in 44 days old leaves all three forms of carbohydrate were lower in the diseased material. The suggestion thus receives support from this fact alone. The general reduction in carbohydrate, however, was not the only difference between the two series, as will be seen by comparing the curves for reducing sugars (Fig. 6). These curves show that reducing sugars in the diseased laminae tended towards greater values during the photosynthetic period, 7.0 a.m. to 7.0 p.m., and towards lower values during the night. The differences in fluctuations of reducing sugars are the reverse of those found in the first diurnal series, and their interpretation must be sought in the light of the differences in metabolism between the leaves sampled at 20 and at 44 days. In the former series the products of photosynthesis were found to be directed mainly towards utilization in tissue synthesis. Reducing sugars, i.e. the basic carbohydrates of this synthesis, pre-

dominated in the leaf. Disturbances in metabolism were reflected directly in the fluctuations of reducing sugars, and the fact that these latter were of smaller values during the day and of greater values at night in the diseased leaves has been taken to indicate that they were formed to a less extent in photosynthesis and utilized to a less extent in general synthesis. In the 44 days old material, however, the picture of metabolism drawn from the carbohydrate relations of the healthy leaf was that of

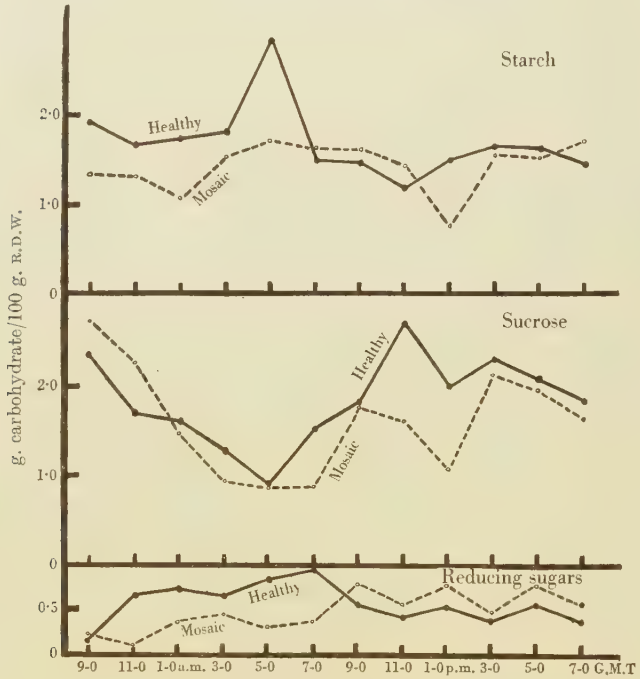

Fig. 6. Diurnal variations in carbohydrates of the leaves of healthy and mosaic President at 44 days.

carbohydrate synthesis directed to the laying down of both temporary and permanent carbohydrate reserves. Sucrose and starch were shown to serve as temporary storage products and to predominate largely over reducing sugars. Consequently, disturbances in metabolism should be most readily observed in the formation of sucrose and starch, i.e. in the main direction of synthesis. The enhanced values of reducing sugars in the diseased leaves during the photosynthetic period is not interpreted as indicative of an increased rate of carbon assimilation, therefore, but of a

reduced rate of formation of sucrose and starch leading to lower values of these latter. Conversely, the lower values of reducing sugars during the night may be used as evidence that the demand for these sugars was greater than the supply made available by hydrolysis of the temporary reserves and hence that the lower values were brought about by an impeded rate of hydrolysis of starch and/or sucrose. Since the starch fluctuations in the diseased laminae tended to lag behind those in the healthy, it would seem that the disturbed relationships were primarily those between reducing sugars and starch.

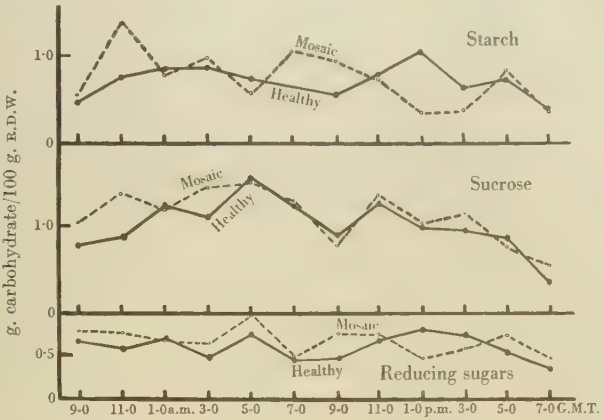

Fig. 7. Diurnal variations in carbohydrates of maturing leaves of healthy and mosaic President at 86 days.

President, 86 days old. The comparative curves for the third diurnal series (Fig. 7) show that reducing sugars and sucrose fluctuated in similar fashion in both healthy and diseased laminae and that starch fluctuated inconsistently with time in the diseased laminae. From the comparison of mean levels (Table VII) it is evident that with approaching senescence the relations of the carbohydrates in the diseased leaf, as compared with those in the healthy leaf, were different from those observed earlier in the season. Reducing sugars and sucrose were of higher values in the diseased laminae, whilst starch values, although lower, were appreciably near those of the healthy leaf. There was, therefore, a tendency towards a relative accumulation of all three carbohydrates in the diseased leaves. This is in complete agreement with previous observations on the maturing leaves of mosaic diseased potatoes (Barton Wright & McBain, 1933*a*; Stone, 1936).

It has already been suggested that the metabolism of the healthy leaf at this stage of development is directed chiefly towards the breakdown of reserve carbohydrates and transport of soluble sugars to the tubers. In these circumstances, the accumulation of soluble sugars and starch in the diseased laminae may be due to an accelerated rate in the hydrolysis of reserve carbohydrates and/or a retarded rate of transport. The fact that reducing sugars in the petioles of the diseased material were significantly lower than in the healthy (Table VII) suggests that there was a marked interference with translocation. This again is in agreement with the findings of Barton Wright & McBain (1933*a*).

Table VII

Mean values	Healthy	Mosaic	Difference	D/E_D
		Lamina		
% R.D.W.:				
Reducing sugars	0·594 ±0·039	0·666 ±0·046	0·072 ±0·060	1·20
Sucrose	1·011 ±0·082	1·120 ±0·083	0·109 ±0·117	0·93
Starch	0·801 ±0·107	0·739 ±0·092	0·062 ±0·141	0·44
% Total:				
Reducing sugars	25·55 ±1·67	26·94 ±1·44	1·39 ±2·21	0·63
Sucrose	41·82 ±1·48	44·40 ±2·25	2·58 ±2·70	0·96
Starch	32·54 ±2·11	28·42 ±2·34	4·12 ±3·15	1·31
		Petiole		
% R.D.W.:				
Reducing sugars	2·689 ±0·234	1·505 ±0·112	1·184 ±0·259	4·57
Sucrose	1·060 ±0·270	0·946 ±0·092	0·114 ±0·285	0·40
Starch	1·086 ±0·102	0·909 ±0·041	0·177 ±0·110	1·61

Seasonal series.

As in the case of the diurnal data, similarity is the most striking feature of the curves showing seasonal variations in the total carbohydrates of the healthy and diseased leaves. There are slight differences between the comparable curves, however, and an examination of these will serve to strengthen the various suggestions arising out of the differences observed in diurnal metabolism.

An inspection of the curves for President (Fig. 8) reveals a tendency towards higher carbohydrate values in the diseased leaves between 20 and 37 days but towards lower values during the greater part of the period of carbohydrate accumulation.

It has been suggested that in the developing leaves of the healthy controls there was a balance between carbon assimilation and carbohydrate utilization in support of growth. Later, when the leaves were fully expanded, the balance was destroyed and carbohydrate formation exceeded its utilization in growth with the result that storage products

were accumulated in increasing quantities. The higher values of total carbohydrate in the diseased leaves at the beginning of the period of accumulation may be taken to indicate, therefore, that the diversion of assimilated carbon from utilization in growth to the laying down of storage products took place earlier in these leaves than in the healthy leaves. The curves show that the carbohydrate content of the latter

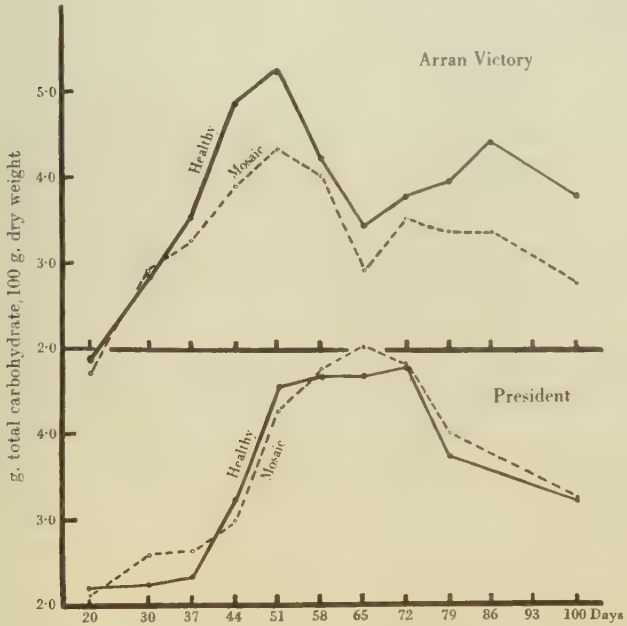

Fig. 8. Seasonal variations in some carbohydrates of the leaves of healthy and mosaic President and Arran Victory.

increased at a greater rate than that of diseased leaves with the result that carbohydrate values were at a higher level in the healthy leaves. This points to a reduced efficiency in carbon assimilation in the diseased leaves and leads to the suggestion that the earlier reversal was caused by a retardation in growth activities within these leaves resulting in a lessened demand for carbohydrate in the support of tissue synthesis.

Increase in the carbohydrate content of the healthy leaves was rapid at first but diminished in course of time with the establishment of a fairly steady maximum at 51–72 days. The carbohydrate content of the

diseased leaves, however, continued to increase after that of the controls had reached its maximum with the result that from 58 days onwards there was a greater amount of carbohydrate in the diseased leaves than in the healthy. There is no reason for assuming that increased photosynthetic activities of the diseased leaves were responsible for the higher carbohydrate content. On the other hand, there is justification for adopting the alternative assumption that diminished utilization of carbohydrate in the diseased plants affected the comparative levels between the two series. It will be remembered that the diurnal series of samples taken at 44 days and 86 days were obtained from plants in their pre-flowering and post-flowering stages, respectively, and it follows that the interval between these samples covered the flowering period of the material under review. There is evidence that infection of President with potato virus *X* is accompanied by a reduction of the number of flower trusses and also by a reduction of the number of flowers formed per truss (unpublished data). Consequently, it may be expected that there would be a lessened demand upon carbohydrate for the maintenance of growth and development of flowers in diseased plants as compared with healthy plants. The data for 86 days old President have been interpreted as showing that there were disturbances affecting translocation which resulted in relatively higher carbohydrate contents in the diseased plants. Impeded translocation, therefore, may also have been a determining factor in the accumulation of carbohydrate in the diseased plants from 58 days onwards. The two factors, impeded transport and reduced flowering capacity, may be causally linked, and the observed effects on carbohydrate economy in diseased plants may be due to either or both. Neither affect sugar formation, however, but both affect its utilization outside the leaf and, therefore, there are grounds for support of the assumption that the higher levels of carbohydrate in diseased leaves during the flowering period are due to diminished demands upon carbohydrate for use outside the leaves.

The higher values of carbohydrate in the diseased leaves during the whole period of carbohydrate decrease with approaching maturity give support to the observations recorded for 86 days old President but throw no further light on the cause of disturbance.

If the seasonal curves for Arran Victory are now examined it will be seen that after a transient reversal in carbohydrate values at 30 days, carbohydrate in the diseased leaves remained at a lower level throughout the season. The trend of fluctuation was the same in both healthy and diseased leaves, however, and the response to manurial treatment was

apparently greater and was maintained over a longer period in the healthy leaves than in the diseased leaves. In consequence the differences in carbohydrate contents of the two series widened considerably towards the end of the season.

Total nitrogen.

The diurnal samples of President taken at 44 and 86 days, the diurnal samples of Arran Victory taken at 30 and 72 days, and each seasonal sample of both varieties were analysed for total nitrogen. In every comparison the diseased material was found to have a higher nitrogen content and the differences of the mean diurnal values (Table VIII) were highly significant. The data in Table VIII indicate the main features of

Table VIII

Variety	Age in days	Total nitrogen as g./100 g. R.D.W.			
		Healthy	Mosaic	Difference	D/E_D
Arran Victory	30	6·829 ±0·060	7·090 ±0·022	0·261 ±0·064	4·08
President	44	6·555 ±0·047	6·875 ±0·051	0·320 ±0·069	4·64
Arran Victory	72	5·546 ±0·056	6·138 ±0·069	0·592 ±0·107	5·53
President	86	5·435 ±0·037	5·867 ±0·040	0·432 ±9·055	7·85

the investigation, namely, that the amount of nitrogen decreased with increasing age of the plant; that nitrogen was greater in the diseased series; and that the differences widened with increasing age of plant. No comparative curves are given, since in every case they were almost parallel and throw no light on the causes of disturbance in nitrogen metabolism.

V. Discussion

It has been emphasized that the comparison between carbohydrate variations, over diurnal and seasonal periods, has established a similarity in the gross metabolism of carbohydrates in normal and mosaic-infected potato leaves. Nevertheless, a detailed examination of the data has provided evidence that modifications of the fundamental metabolism, slight though definite in character, may arise in consequence of mosaic infection. At each stage of diurnal investigation there were lower starch values in the diseased laminae, and slight but noticeable differences in carbohydrate variations pointed consistently towards impediment in starch formation and hydrolysis. In addition, there was indication of an interference with the utilization of sugars during the early stages of growth when the leaves were actively engaged in tissue synthesis. This interference became pronounced later, and it was suggested that the

cause was to be found in a premature retardation of growth activities which was reflected in the diversion of the products of photosynthesis from utilization in tissue synthesis to the formation of storage carbohydrates. This change in metabolism proceeded in normal fashion and merely anticipated a similar change in the healthy leaf. The difference in trend of metabolism between the two series was, moreover, transient, and subsequently the only differences to be found were in slightly reduced amounts of carbohydrate and in the persistent interference with starch elaboration in the diseased leaves. A further apparent disturbance was noted during the flowering period of growth in President, but again, though the evidence was indirect, the cause was sought not in carbohydrate metabolism *per se* but in its relations with the general metabolism of flower production.

During the pre-senescent stages of growth an interference with translocation became evident. In consequence there was a slight disorganization of metabolism due to the accumulation of soluble sugars, but no trace was found of any large interference with the normal processes of carbohydrate degradation characteristic of the period.

The causation of these slight disturbances in the carbohydrate economy of mosaic-infected potato leaves may be sought in two possible sources. In the first place, the slightly reduced efficiency in carbon assimilation, the disturbed starch-sugar relationships, and the impeded translocation, may be traced directly to the pathological changes brought about by the virus. Smith (1924) and Clinch (1931) have made anatomical studies of potato leaves affected with mosaic diseases, and their observations show that histopathological symptoms within leaves affected with mild or simple mosaic are, in general, restricted to the mottled areas. In these areas the mesophyll is undeveloped, there is a reduction in size and number of chloroplasts, and the chlorophyll has a paler appearance. Clinch has shown further that the extent of these modifications is proportional to the external visible symptoms of the disease and that there are marked abnormalities in the starch content of cells in the chlorotic areas. In contrast with the evidence of the above authors, Rochlin (1930) has stated that pathological symptoms consequent upon infection with mosaic viruses are not restricted to the mottled areas but may spread to destructive changes in the conducting tissues. These conclusions are qualified, however, by the observation that the extent of internal symptoms is correlated with the severity of the external symptoms.

Since the disease symptoms upon the leaves of the two potato varieties used in the present investigation were confined to small, slightly

chlorotic, mottled areas, and in view of the anatomical evidence the slight disturbances which have been observed in diurnal carbohydrate metabolism would appear to be directly associated with the presence of the mottled areas. It would seem that the greater portion of the leaf surface functions normally with regard to the formation and utilization of sugars, resulting in a gross similarity between observations for normal and diseased leaves, but that in the relatively small areas in which pathological symptoms are manifested the formation of sugars in photosynthesis does not take place, or takes place to a small extent only, and the formation and degradation of starch are subject to impediment. This conclusion is in agreement with that of Cook (1926) who has shown that in sugar cane the green areas of mosaic-infected plants synthesize sugars in the normal manner but that the products of photosynthesis, particularly starch, are reduced in proportion to the amount of visible infection.

The interference with translocation towards the end of the season may, in the light of Rochlin's evidence (1930), be ascribed to pathological effects, but in the absence of specific data upon the channels through which reducing sugars are removed from the leaves, no great stress can be laid upon this suggestion.

A second cause of modification in the carbohydrate metabolism of mosaic-infected leaves has been traced to disturbances affecting primarily the growth activities of the plant. Thus, in the early stages of development, indication was obtained that growth was retarded in the diseased leaf earlier than in the healthy leaf. During the flowering stage, also, there was indication that carbohydrate was not used in support of flower production to the same extent as in the healthy plant. There has been no evidence to suggest, however, that disturbances in carbohydrate metabolism were the causes of these interferences with growth. It would seem that they were effects and that the causes must be sought in some other aspect of general metabolism.

The obligate parasitism of viruses suggests that their auto-synthesis is dependent upon the partly synthesized intermediate products of metabolism and upon the physiological conditions extant in living tissue. More particularly, in view of the protein nature of mosaic viruses (Stanley, 1935), it would seem that the metabolism of nitrogen would provide a suitable source of materials for this synthesis. On this hypothesis the pathological symptoms and retarded growth activities which give rise to disturbances in carbohydrate metabolism may well be direct manifestations of a disorganized nitrogen metabolism.

VI. Summary

The effects of potato virus *X* upon the carbohydrate and total nitrogen contents of potato leaves have been followed over diurnal and seasonal periods in the varieties President and Arran Victory.

The gross metabolism of carbohydrates has been found to be similar in both mosaic-diseased and normal leaves.

Three major trends of carbohydrate metabolism, each of which may be identified with a particular stage of leaf development, have been recognized and discussed.

Slight modifications of the fundamental metabolism of diseased leaves have been found in a reduced photosynthetic activity, disturbed starch-sugar relationships, and an impediment in translocation during the pre-senescent stage. All these have been ascribed to secondary effects arising out of the pathological change brought about as a result of virus infection.

Transient modifications in carbohydrate metabolism have been traced to prematurely retarded growth activities leading to a diminished demand for carbohydrate in support of growth in the diseased plants.

A significantly greater content of nitrogen has been found in diseased leaves at all stages of growth, and it has been suggested that the pathological symptoms and prematurely retarded growth activities which give rise to disturbances in carbohydrate metabolism are direct manifestations of a disorganized nitrogen metabolism.

References

Barton Wright, E. & McBain, A. (1932). Studies in the physiology of the virus diseases of the potato. *Trans. roy. Soc. Edinb.* **57**, 309–49.

—— —— (1933*a*). Studies in the physiology of the virus diseases of the potato. II. *Ann. appl. Biol.* **20**, 525–48.

—— —— (1933*b*). Studies in the physiology of the virus diseases of the potato. III. *Ann. appl. Biol.* **20**, 549–89.

Blackman, V. H. (1919). The compound interest law and plant growth. *Ann. Bot., Lond.*, **33**, 353–60.

Bunzel, H. H. (1914). Oxidases in healthy and in curly dwarf potatoes. *J. agric. Res.* **2**, 373–404.

Clements, H. F. (1930). Hourly variations in carbohydrate content of leaves and petioles. *Bot. Gaz.* **89**, 241.

Clinch, Phyllis (1931). Cytological studies of potato plants affected with certain virus diseases. *Sci. Proc. roy. Dubl. Soc.* **20** (N.S.), 143–72.

Cook, M. T. (1926). Photosynthesis of the sugar cane mosaic plant. *J. Dep. Agric. Porto Rico*, **10**, 239–42.

Davis, W. A., Daish, A. J. & Sawyer, G. C. (1916). Studies of the formation and translocation of carbohydrates in plants. I. *J. agric. Sci.* **7**. 255–326.

DAVIS, W. A. & SAWYER, G. C. (1916). Studies of the formation and translocation of carbohydrates in plants. III. *J. agric. Sci.* **7**, 352–84.

FISHER, R. A. (1932). *Statistical Methods for Research Workers.* London.

LINK, K. P. & TOTTINGHAM, W. E. (1923). *J. Amer. chem. Soc.* **45**, 439.

MASON, T. G. & MASKELL, E. J. (1928). Studies on the transport of carbohydrates in the cotton plant. I. *Ann. Bot., Lond.*, **42**, 1–65.

MILLER, E. C. (1931). *Plant Physiology.* New York and London.

ONSLOW, M. W. (1931). *The Principles of Plant Biochemistry.* Cambridge.

PEARSALL, W. H. & WRIGHT, A. (1929). The proportions of soluble and insoluble nitrogenous materials in fresh and dried plant tissue. *Proc. Leeds phil. lit. Soc.* **2**, 27–31.

PRIESTLEY, J. H. (1929). Cell growth and cell division in the shoot of the flowering plant. *New Phytol.* **28**, 54–81.

RANKER, E. R. (1925). Determination of total nitrogen in plants and plant solutions: a comparison of methods with modifications. *Ann. Mo. bot. Gdn*, **12**, 367–80.

ROCHLIN, E. (1930). Zur Anatomie der mosaikkranken Kartoffelpflanzen. *Phytopath. Z.* **2**, 455–68.

SCHAFFER, E. A. & HARTMANN, A. F. (1921). The idiometric determination of copper and its uses in sugar analysis. *J. biol. Chem.* **45**, 365.

SMITH, K. M. (1924). On a curious effect of mosaic disease upon the cells of the potato leaf. *Ann. Bot., Lond.*, **38**, 150.

STANLEY, W. M. (1935). Isolation of a crystalline protein possessing the properties of tobacco mosaic virus. *Science*, N.S. **81**, 644–5.

STONE, W. E. (1933). Normal growth of potato leaves in greenhouse and field. *J. agric. Res.* **46**, 565–78.

— - (1936). Growth, chemical composition and efficiency of normal and mosaic potato plants in the field. *J. agric. Res.* **52**, 295–309.

WENT, F. A. F. C. (1898). Chemisch-physiologische Untersuchungen über das Zuckerrohr. *Jb. wiss. Bot.* **31**, 289–344.

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OBSERVATIONS ON APPLE CANKER

I. THE DISCHARGE AND GERMINATION OF SPORES OF *NECTRIA GALLIGENA* BRES.[1]

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(With Plate XXIII and 4 Text-figures)

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Introductory

The term apple canker is loosely used to include the lesions resulting from attacks of a number of fungi (Ogilvie, 1935), or from severe woolly aphis attack, or from certain physiological disorders producing similar symptoms. It is applied to almost any kind of open wound surrounded by more or less necrotic tissue. The observations described in this paper have been confined to the canker disease due to *Nectria galligena* Bres.,

[1] Read before the Association on 9 December 1938.

termed in America European canker to distinguish it from similar cankers having their origin in the attacks of other parasitic fungi.

The disease is common in the European countries and is now established in the north-western and north-eastern fruit-growing areas of U.S.A. and in eastern Canada (Dickson, 1925; Zeller, 1926). In addition to causing cankers which may in time girdle and kill a branch, the fungus also causes a calyx- or "eye"-rot of apples and pears (Dillon Weston, 1927; Osterwalder, 1931).

In the study of any disease, one of the chief factors to be considered is the prevalence of infective material. The purpose of this investigation has therefore been to examine the extent to which spores of the fungus are present under natural conditions throughout the year. Two spore stages of the fungus are recognized as responsible for the spread of the disease, viz. macroconidia and ascospores. The macroconidia are produced in very large numbers in small, dirty white sporodochia which push through the killed bark of the host, not infrequently in roughly concentric circles round the point of infection. It is usually this stage which first becomes apparent on the lesions appearing in spring and summer on one- and two-year-old wood, often ringing these young growths.

The macroconidia are followed later in the season by the winter stage, appearing as irregular groups of red perithecia, from the apical pores of which large numbers of ascospores are liberated. The perithecia are similar in colour to the eggs of red spider, but are about twice the diameter of the latter and differ also in that they are confined to cankered bark.

THE CAUSAL ORGANISM

Historical

European canker is caused by a fungus of the genus *Nectria*, though agreement was not reached for some time concerning the species responsible. Historical reviews of the mycological literature on the subject are given by Cayley (1921) and Zeller (1926). Zeller concludes that the earlier work deals with two or possibly three separate organisms.

In 1865 Tulasne described *N. ditissima* from beech, and this organism was subsequently shown to be the cause of canker in beech (Hartig, 1880) and in apple (Goethe, 1904). The conidial stage of the fungus, first described by Willkomm in 1866, also from beech, as *Fusidium candidum*, was transferred by Lindau (1909) to *Fusarium Willkommii*.

Nectria ditissima Tul. was accepted by von Höhnel & Weese (1910) as synonymous with *N. coccinea* (Pers.) Fr. Weese (1911), working with *N.*

ditissima Tul., *N. coccinea* (Pers.) Fr., and *N. galligena* Bres. (Bresadola, 1901), came to the conclusion that canker in fruit trees and in timber trees is due to *N. galligena* Bres., which he distinguished from *N. ditissima* Tul. by differences in the structure of the perithecium, in the characters of the spores, and chiefly as a canker producer. *N. galligena* Bres. he found associated with definite cankers, whereas the other two species were confined to smooth areas of bark. Weese confirmed the statement of Appel & Wollenweber (1910) that *Fusarium Willkommii* Lindau was the conidial stage of *Nectria galligena* Bres. Wollenweber (1913*a*) also attributes canker in apple trees to *N. galligena* Bres. (= *Fusarium Willkommii* Lindau).

Wollenweber (1913*b*) segregated those forms having macroconidia with rounded ends from the genus *Fusarium* Link as a distinct genus *Cylindrocarpon* Wr., and the conidial stage of *Nectria galligena* Bres. was transferred from *Fusarium* (= *Cylindrocarpon*) *Willkommii* to *Cylindrocarpon mali* (Allesch.) Wr. = *Fusarium mali* Allesch. (1892). The name *Cylindrocarpon Willkommii* (Lind.) Wr. was given to the conidial stage of *Nectria ditissima* Tul. This ascription has generally been accepted by later workers, who include Cayley (1921) in this country, Zeller (1926) in Oregon, U.S.A., and Richter (1929) on the continent.

Other important papers in recent years add further points. Westerdijk & van Luijk (1924), from investigation of the fungi in pure culture, distinguish between *N. galligena* Bres. and *N. coccinea* (Pers.) Fr. on the basis of differences in ascospore dimensions—17–18 and 12–13 μ in length respectively. They are of the opinion that if the biometric method of ascospore measurement were adopted, *N. ditissima* Tul. would fall into one of these two species. The characters of the perithecial wall and of the macroconidia they find too variable to serve as reliable criteria for classification.

Richter (1929) disagrees and claims that the conidial characters are of importance in distinguishing between *N. galligena* Bres. and *N. ditissima* Tul. By plotting the frequency curves for ascospore length, a sharp difference is found between the steep curves for four strains of *N. coccinea* (Pers.) Fr. and the much flatter curves for two strains of *N. galligena* Bres. The curves for *N. ditissima* Tul. and *N. punicea* (K. & Schm.) Fr. are intermediate in form. These two species are separated by differences in the ratio of length/breadth of the ascospores. Conidial characters also differ in the four species. The conidia of *N. ditissima* Tul. are longer and more slender (4·9 μ) than those of *N. galligena* Bres. (5·6 μ), which, like those of *N. punicea*, are also more curved. Inoculation experiments

showed that *N. galligena* would readily produce typical cankers on apple, less readily on pear and beech, while *N. ditissima* gave cankers on all three. *N. coccinea* and *N. punicea* proved non-pathogenic in Richter's experiments.

Moritz (1930) reports inoculation experiments with twenty-two strains of *Nectria* spp. His results emphasize the existence of strains of *N. galligena* Bres. differing in virulence, some being able to produce cankers on a range of host trees, including apple, while others were entirely non-pathogenic. Moritz differs from Richter in finding that *N. coccinea* (Pers.) Fr. is capable of causing canker of apple shoots, and finds no evidence of host specialization.

Wiltshire (1921), in an account of the method of infection by the Apple Canker fungus through leaf scars, described it under the name of *N. ditissima* Tul. though acknowledging that doubt existed as to the correct nomenclature. Cayley (1921), from a study of the fungus, chiefly in pure culture, considered that the organism causing canker on Apple in England was *N. galligena* Bres. No comprehensive measurements of the spores of the fungus are given, however, to provide a comparison with the descriptions of Wollenweber (1913*b*, 1929) and Richter (1929).

Descriptive

(1) *Macroconidia.*

In the present investigation, conidia from pure cultures of different strains of the fungus on various media have been measured. The media used have been malt-extract agar, oatmeal agar, and wheatmeal agar; while, for comparison, conidia have also been measured from cankered bark kept in moderately damp conditions in the laboratory. All spores have been measured in distilled water, care being taken to discontinue measurement before the result was affected by the onset of germination, which, at laboratory temperatures, may be perceived after about 3 hr. The media used had little influence on the dimensions of the macroconidia of similar septation. Malt-extract agar and oatmeal agar tended to give conidia of fewer septa than those developing on wheatmeal agar, which very closely resembled the conidia developing in the laboratory on naturally cankered bark.

The mean results of the measurements of macroconidia of three strains of the fungus are assembled in Table I. The strains were isolated as follows:

Strain 1. From a canker on apple resulting from natural infection of a pruning cut. Monoconidial isolation.

Strain 2. From a large superficial canker apparently of recent development and almost girdling a large branch of apple.

Strain 3. From a leaf scar infection of apple. Monoconidial isolation.

Table I. *Comparison of spore measurements (in μ)*

Nectria galligena Bres.

Macroconidia. *Cylindrocarpon mali* (Allesch.) Wr.

Strain	1-septate	2-septate	3-septate	4-septate	5-septate	6-septate	7-septate
Strain 1:							
Wheatmeal agar	—	—	35 × 5·0	43 × 5·4	61 × 5·6	—	—
Oatmeal agar	25 × 4·0	—	40 × 5·0	51 × 5·3	59 × 5·6	—	—
Cankered bark	22 × 4·0	28 × 4·4	38 × 4·8	45 × 5·0	54 × 5·4	60 × 5·6	—
Strain 2:							
Wheatmeal agar	22 × 3·9	—	—	45 × 5·3	61 × 5·6	69 × 5·9	71 × 6·0
Malt-extract agar	—	—	39 × 5·2	50 × 5·4	52 × 5·4	—	—
Strain 3:							
Wheatmeal agar	—	—	—	—	56 × 5·4	—	—
Malt-extract agar	24 × 4·5	29 × 4·8	39 × 5·3	48 × 5·4	—	-	—
Authority							
Wollenweber, 1913	—	—	—	48–50 × 4·8–5·8	54–62 × 5–6	68 × 6	68 × 6
1929	—	—	41 × 5·0	—	56 × 5·5	—	—
Richter, 1929	—	—	38 × 5·0	47 × 5·2	60 × 5·5	68 × 5·8	76 × 5·75

Nectria ditissima Tul.

Macroconidia. *Cylindrocarpon Willkommii* (Lind.) Wr.

Authority							
Wollenweber, 1929	—	—	37 × 3·5	—	72 × 4·4	—	89 × 4·4
Richter, 1929	—	—	—	—	69 × 4·9	77 × 4·9	85 × 4·8

The measurements given for *Cylindrocarpon mali* (Allesch.) Wr. by Wollenweber (1929) and Richter are included for purposes of comparison, as also are those for *C. Willkommii* (Lind.) Wr., the conidial stage of *Nectria ditissima* Tul. The conclusion is drawn that the conidial stage of the fungus isolated at Long Ashton is the same organism as that described by Wollenweber (1913*b*, 1929) and by Richter (1929) as *Cylindrocarpon mali* (Allesch.) Wr.

It was sometimes observed that a strain of the fungus, after about six months of pure culture, ceased to produce typical macroconidia. The spores then produced were short and possessed few or no septa. Typical macroconidia, on the other hand, have been formed in large numbers in strains of the fungus after culture for nearly two years.

(2) *Ascospores.*

Perithecia were produced in pure culture on potato glycerine as described by Cayley (1921). The ascospores extruded were not suitable for measurement, since in most cases they had already germinated.

A number also were single-celled with few cell contents. Ascospores from perithecia developing on cankered bark under natural conditions have therefore been measured as described for macroconidia. The figures so obtained agree with those described for *Nectria galligena* Bres. The other characters of the fungus support this conclusion:

Perithecia: red, ovoid-pyriform, 300–450 × 250–350 μ, occurring singly or in groups. A stroma is not invariably apparent. Ostiole raised and consisting of radially arranged cells, of slightly darker colour. Asci: clavate; 8-spored, one spore occasionally abortive, 1- or 2-seriate, 85–120 × 10–16 μ. Ascospores: hyaline, 2-celled, often with constriction at the septum. Mean dimensions: 16·3 × 7·5 μ. Extremes: 11·5–22 × 5·5–9·2 μ.

Macroconidia: hyaline, 5–7-septate, allantoid. Abstricted from branched conidiophores aggregated into dirty-white sporodochia. Anastomoses frequent in old sporodochia. 3-septate, 39 × 5·1 μ; 4-septate, 47 × 5·3 μ; 5-septate, 60 × 5·6 μ; 6-septate, 69 × 5·9 μ; 7-septate, 71 × 6 μ.

Mycelium: hyaline with reddish stromata becoming dark brown. Inoculation of apple trees with pure cultures produced typical cankers.

(3) *Cultural characters.*

The fungus grows readily on the common media, forming much white aerial mycelium. After 15–20 days, cultures on malt-extract agar, potato-dextrose agar, and especially on oatmeal agar, developed plectenchymatous stromata which became dark reddish brown in colour. Wheat-meal agar produced very little mycelial growth, but conidial sporodochia appeared in a few days clustered around the inoculum. In appearance they closely resembled the sporodochia developing naturally on cankered bark. Similar sporodochia were formed on other media in the aerial mycelium, though their appearance was delayed in the cultures on potato glycerine (Cayley, 1921).

Measurement of the diameters of colonies of the fungus in pure culture on malt-extract agar at temperatures ranging from 2 to 37° C. showed that the optimum temperature for growth is about 20° C. Growth is slow and the hyphae stunted at 30° C. and the fungus is killed at 37° C. Growth is slow but appreciable at 2° C., and at this temperature the diameter of the colonies increased by about 0·8 mm. per day.

In pure cultures of the fungus on sterilized pieces of apple bark, conidial sporodochia developed and in course of time assumed the same colour as naturally occurring perithecia. No perithecia, however, were produced and the sporodochia thus modified were, at this stage, not able to form viable macroconidia. Structures resembling perithecia in size and colour were found in a Petri-dish culture of *N. galligena* on malt-extract

agar after about 3 weeks, but remained sterile. True perithecia with asci and ascospores developed in pure culture on the potato-glycerine medium (Cayley, 1921). The ascospores were found extruded at the ostioles of the perithecia, and many had germinated.

Anastomoses of conidia through short conjugation tubes as described by Cayley (1921) were of frequent occurrence in the sporodochia of older cultures. A similar though less frequent fusion was observed in the germ tubes of macroconidia germinating in hanging drops in distilled water.

(4) *Seasonal spore development.*

During late winter or early spring many small cankers may develop by infection at the leaf scars of the previous season's wood, as described by Wiltshire (1921). During a moderately warm spell in March or April, these small cankers may show the conidial sporodochia. These burst through the epidermis as dirty white circular cushions 1–2 mm. diameter, frequently arranged in roughly concentric arcs round the source of infection. Like the similar but looser sporodochia formed in culture, these consist of closely branching hyphae, from the tips of which the characteristic spores are abstricted. Sporodochia have been observed before the middle of March on young cankers resulting from leaf-scar infections. The conidia from these sporodochia were mostly two-celled however in place of the typical 5- or 6-septate spores.

Except in rather damp conditions, few sporodochia are produced during the summer months, but renewed activity is apparent in September and early October. The most favourable conditions are a warm humid spell following rain. True sporodochia are not formed after November, although macroconidia with 3–5 septa have been collected in small numbers throughout the year on vaselined glass slides.

Lower temperatures are followed by the appearance of the red perithecia, which at first develop at the edges of the sporodochia. The numbers of perithecia increase from October onwards, being at a maximum during December. Mature asci and ascospores are found on sectioning perithecia at the end of October or early November. Zeller (1926) for Oregon, U.S.A., conditions describes the time of maturing of the perithecia as the three months following the autumn rains. A continuous humid atmosphere from the beginning of October onwards resulted in spore discharge at the end of November or beginning of December. A dry autumn, however, caused perithecial development to be delayed, so that spore discharge was not observed until the first week in January.

Low temperature alone is not responsible for the formation of perithecia. Cultures of the fungus on sterilized pieces of apple bark were kept out of doors during the autumn and early winter of 1936. The conidial sporodochia became coloured in the same way as the perithecia of *N. galligena* but no trace of perithecial structures was observed on sectioning. Macroconidia were present but incapable of germination. Perithecia were found by Cayley (1921) to develop in culture on a medium rich in starch and in the presence of glycerine. Under natural conditions perithecia are much more numerous on old-established cankers than on small cankers on young wood.

In December, and especially later in the winter, white masses of ascospores may be observed at the ostioles of the perithecia. These masses, as they dry out, become yellowish in colour. They may appear on perithecia kept in moist conditions in the laboratory. Zeller (1926) has suggested that they arise from the simultaneous discharge of several asci blocking the ostiole of the perithecium. If the masses are removed, as by washing, normal ascospore discharge may be renewed for a time. Sections of perithecia bearing such spore masses frequently show more or less disorganization of the asci, which may be absent, ascospores with branching germ tubes lying free within the perithecium. It would appear, therefore, that the appearance of masses of ascospores at the ostiole may be an indication of the exhaustion of the perithecium.

Spore dissemination

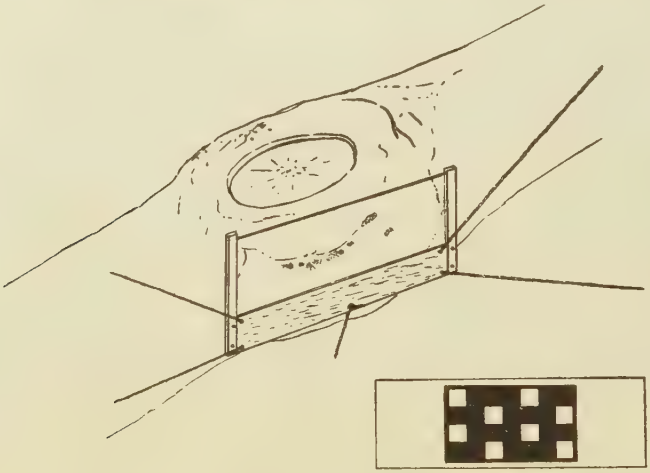
Ascospore discharge

(1) *Mode of discharge.*

As stated above, the present investigation has been directed towards determining the output of spores from the fructifications of the fungus throughout the year. Attention has been chiefly focused on the ascospores. Most accounts of the fungus in the past have described discharge of the ascospores from the perithecia by extrusion from the ostioles as small masses or tendrils. Wiltshire (1919), however, mentioned that it was convenient to establish pure cultures of the fungus directly from ascospores ejected on agar plates from perithecia placed below.

It has been observed that the ascospores of the fungus may be ejected from the perithecia with considerable force. The eight spores from each ascus are typically ejected simultaneously as a single mass which may be collected on a cover-glass or microscope slide (Pl. XXIII, fig. 1). Many separate groups, each containing eight ascospores, may thus be

deposited. Such groups have been collected in the laboratory on cover-glasses supported on glass rings at distances of up to 3 cm. vertically above perithecia of *N. galligena*. The perithecia employed were growing on detached portions of cankered apple bark placed on wet filter paper. Zeller (1926), in Oregon, also describes this forcible ejection of ascospores of *N. galligena* from the perithecia. Observations have demonstrated that under field conditions also, dispersal of the ascospores occurs predominantly by the forcible discharge of the eight spores of the ascus



Text-fig. 1. Slide carrier and counting plate.

simultaneously. Figs. 1 and 2, Pl. XXIII, were photographed from vaselined slides used in the field work as described below, and fig. 2 shows clearly how the spores from two asci have crossed on the slide.

(2) *Field investigations.*

The perithecia of the fungus develop, frequently from the margin of the sporodochia, during the early autumn, becoming more numerous as winter approaches. Cayley (1921) follows previous workers in describing the perithecia as maturing during the winter and dehiscing in spring. Accordingly, preliminary spore-trapping experiments were started in the spring of 1936 in the plantations of Long Ashton Research Station.

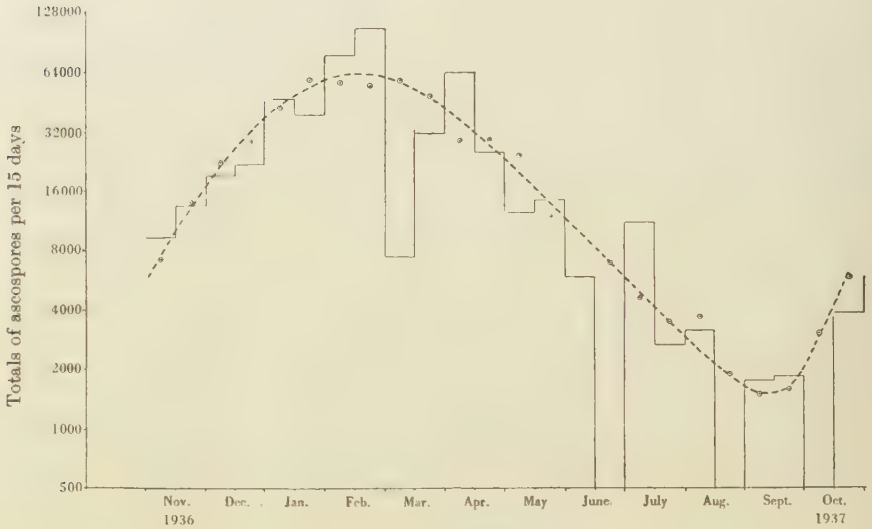
(*a*) *Methods.* Vaselined 3 × 1 in. microscope slides were suspended on edge in suitable carriers (Text-fig. 1) close to cankers showing a number of perithecia. After suitable intervals two or more days—the exposed

slides were brought into the laboratory, being replaced by fresh vaselined slides. The number of ascospores of *N. galligena* present in an area on each slide of $2 \times \frac{7}{8}$ in. was counted after adding sufficient aqueous solution of a proprietary wetting agent to allow clear visibility when a cover-glass was placed over the vaseline film. Note was also kept of the amount of daily rainfall. This procedure was maintained from March 1936 until the middle of May. The results showed that, in general, dispersal of ascospores from the perithecia was dependent on rainfall, but since the numbers fell away steadily from an initial maximum, it was decided to repeat the experiment the following year, commencing observations rather earlier.

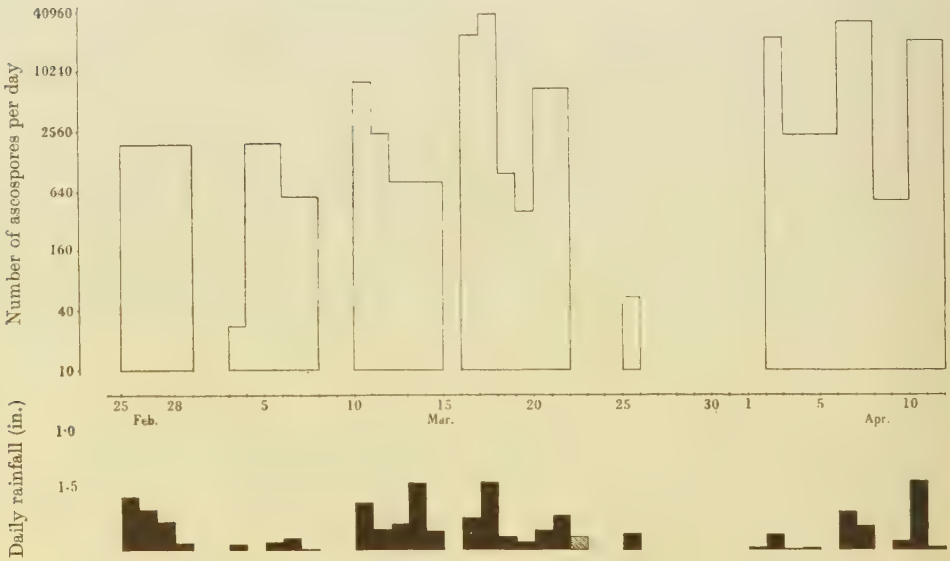
In order to permit of more frequent counts of the ascospores, small squares distributed over the exposed vaselined slides were searched. This was achieved by superimposing the exposed slides on an engraved microscope slide placed on the microscope stage. This engraved slide was divided into 5 mm. squares, eight of which were searched. Text-fig. 1 explains their distribution. The total number of spores counted on each slide therefore represents those on an area of 2 sq. cm. If the number of ascospores exceeded about 800 per 5 mm. square, it was found advantageous to estimate the total from counts made on smaller squares delimited by means of a cross-hatched disk in the eyepiece of the microscope.

(*b*) *Records of seasonal and daily discharge.* Observations were commenced soon after the middle of October 1936 in the same plot of trees (var. Lane's Prince Albert) as had been used in the preliminary experiments. Four stations were selected for slides at distances of from 1·5 to 3 cm. from cankers showing numerous perithecia. After being exposed for one or more days, the four slides were replaced with fresh vaselined slides and taken to the laboratory for counting. Slides were changed in this way at 11 a.m., and the air temperature simultaneously recorded at the same height above ground as the cankers. Wet-bulb temperatures close to one of the cankers were also recorded, but the measure of humidity so obtained showed no relation to ascospore discharge. Daily rainfall was also recorded.

Recording was continued with four slides from October 1936 to June 1937, and thereafter with three slides until November 1937. The results are shown in Text-figs. 2 and 3. Text-fig. 2 represents the totals for 15-day periods from October 1936 to October 1937 of the ascospores of *N. galligena* collected on the slides from two of the slide stations recorded. The numbers of ascospores counted on the other slides show a similar relation to the time of year, but are not included since it was not found



Text-fig. 2. Summarized records of ascospore discharge.



Text-fig. 3. Detailed records of ascospore discharge.

possible to maintain their records with sufficient consistency. The 15-day totals are shown as a stepped curve. In an effort to smooth out minor variations resulting from short wet periods or small temperature changes, means have been calculated for a series of 75-day periods, the middle point of each period being 15 days later than that of the preceding period. For each period the figure taken has thus been the arithmetic mean of the five 15-day totals included, and is plotted on the curve at the date representing the middle of the 75-day interval. Thus, the third point represents the arithmetic mean of the five 15-day totals extending from the beginning of November to the middle of January, the fourth, of the five totals from mid-November to the end of January, and so on. These means lie very near a smooth curve showing a distinct optimum period for ascospore discharge during the month of February, the numbers thereafter falling to a minimum during September, and then rising more steeply through October and November.

Over shorter intervals of time the numbers of ascospores collected show a close correlation with the presence or absence of rainfall. The detailed records for the period 25 February to 12 April 1937 are represented in Text-fig. 3, expressed as the totals for the four slides for each 24 hr. period. Where the slides were not changed daily the figures are given as the arithmetic mean per 24 hr. over the period of the exposure. There appears a very close correlation of presence of rainfall with ascospore discharge. In only one case was precipitation in excess of 0·02 in. not attended by discharge of ascospores, viz. from 22 to 23 March. It should be mentioned that, for this interval, the 0·11 in. recorded consisted almost entirely of snow accompanied by low temperatures, and thus the precipitation recorded on the graph for this date does not represent true rainfall. The actual numbers of ascospores discharged show no strict relation to the amount of rainfall for a particular interval of time, though the fact that such a relation exists is strongly suggested, bearing in mind the conditions of the experiment. A number of factors remain uncontrolled. These include, first, the actual time of wetting of the perithecia, which is influenced by wind direction, period of rainfall and relative humidity of the atmosphere, and, secondly, changes in the number of mature perithecia, and exhaustion resulting from prolonged discharge of ascospores.

(3) *Laboratory investigations.*

It was not possible to assess the effect of temperature on ascospore discharge under field conditions. At no time did rain fall when the

temperature was below 5° C. An attempt was therefore made to determine from laboratory experiments if ascospores were discharged at low temperatures. Groups of 30–40 perithecia of *N. galligena* (mature as far as could be judged) on small pieces of cankered bark were wetted and enclosed in Petri dishes at constant temperatures. The ejected ascospores were collected on the under side of cover-glasses supported a few millimetres above the perithecia by glass rings. At intervals the cover-glasses were removed in order to count the ascospores collected, fresh cover-glasses being substituted. The results indicated that, at 2° C., ascospore discharge is considerably reduced, by comparison with that at ordinary temperatures.

Groups of perithecia on cankered bark were wetted and left, as described, overnight at a constant temperature of 2–3° C. After 24 hr. seventy-five ascospores were counted. The same perithecia were then transferred to 18° C., when in 4 hr. over 1600 spores were collected. A similar experiment with perithecia at 2, 5 and 11° C. showed that discharge of ascospores was perceptibly slower at 2 and at 5 than at 11° C. Discharge may continue from a group of perithecia fairly uniformly over 48 hr.

Discharge of conidia

Naturally occurring sporodochia of *N. galligena* consist of closely interwoven branching hyphae, from the tips of which the conidia are produced. Under dry conditions they are compact structures of almost horny consistency, but in contact with water they swell rapidly, liberating many hundreds of conidia. No extensive observations of the dissemination of macroconidia of the fungus have been made, but parallel with the counts of ascospores collected on vaselined slides the numbers of macroconidia have also been counted. The numbers declined from an initial maximum in October 1936, and, as with the ascospores, no conidia were collected in absence of rainfall.

Macroconidia were rarely seen singly on slides placed below and beside cankers bearing sporodochia, but in groups whose disposition on the slide indicated that they had arisen by drops of water striking the slide. It is probable that, apart from being washed down the branch, such splashing represents the only method of dissemination of macroconidia. Wiltshire (1914) found that while woolly aphids carried conidia of the canker fungus, inoculation of the fungus through this agency was unsuccessful.

Spore germination

Ascospores

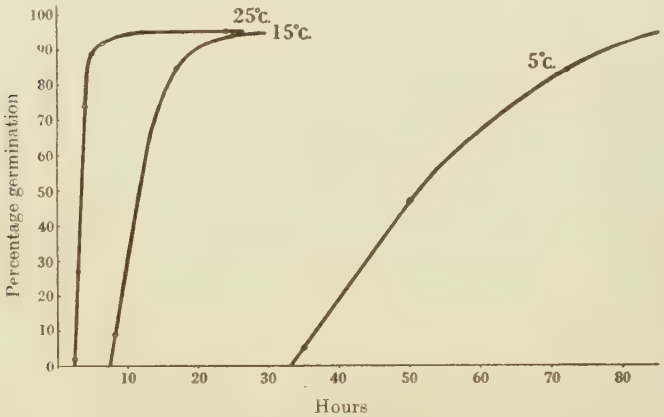
The discharged ascospores are not enclosed in any kind of membrane. The contents of the ascus, with which they are ejected, are readily dispersed in water, but in the absence of a film of water, this material dries, fastening the spores to the substratum, from which they may then be washed only with difficulty. Ascospores collected on a clean glass surface and allowed to remain out of doors for 24 hr. are not removed completely by a vigorous washing under the tap.

The ascospores germinate rapidly in distilled water or nutrient media. Germ tubes are pushed out from the apex of one or both cells of the spore, or they may arise laterally. Germination has most conveniently been examined in the ascospores collected on cover-glasses, as described in connexion with the work on ascospore discharge. These cover-glasses were then transferred when desired, to be placed, spores downward, on glass rings in Petri dishes with damp filter paper. In the course of a few minutes water condensed on the lower side of the cover-glasses in sufficient amount to produce small hanging drops which grew round the spores.

In this way spores were obtained undamaged and, excluding the influence of the host plant, comparable with natural infective material. Germination will occur under such conditions through a temperature range of 2–30° C. Above 30° C. the spores are killed: at 25° C. spores germinate readily but the germ tubes are shorter than those appearing at 20° C. In many cases germination of the ascospores was about 95 % or more. The percentage was occasionally lower, however, owing to the presence of ascospores which were probably immature or abortive when discharged from the ascus. Not infrequently such spores were devoid of septa and had little or no protoplasm.

Counts were made, by the method indicated above, of the percentage germination of ascospores of the fungus at 25, 20, 15 and 2° C. The results are indicated in Text-fig. 4 as the means of a number of percentages, each of 100–300 ascospores. At 25° C. germination commenced in 2–3 hr. and was complete in 6–8 hr. At lower temperatures the rate of germination was reduced, though at 2° C. no actual inhibition of germination occurs. The final percentage of spores germinating at 2° C. is reached only after 4–5 days, but is as great as at higher temperatures. At 15° C. germ tubes up to 300 μ in length were present after 24 hr.

The effect of desiccation on the germination of ascospores was tested by allowing the cover-glasses, with the spores adhering, to remain, protected from dust, in the atmosphere of the laboratory. At intervals these were set up for germination as described above. The few observations made indicated that the resulting percentage germination falls off steadily to zero after desiccation for 5–6 days under these conditions. Similarly, the percentage germination in the ascospores occurring in the spore masses at the ostioles of perithecia is usually less than the figure for freshly discharged ascospores.



Text-fig. 4. Germination of ascospores of *Nectria galligena*.

Conidia

The macroconidia also germinate readily in distilled water and in nutrient media. Germ tubes appear at first usually from one or both terminal cells, though all the cells may ultimately show one or more germ tubes. With macroconidia from sporodochia on apple bark kept in moderately damp conditions germination may reach 100 %. Attempts were made, by adding small portions of the cortex of suitable apple varieties to the water in which macroconidia were germinating, to discover if resistant varieties exercised any inhibiting influence on spore germination. Cox's Orange Pippin, Lane's Prince Albert and Kingston Black (canker-susceptible varieties) were compared in this way with Bramley's Seedling and Sweet Alford (canker-resistant). Control conidia in distilled water gave a mean germination percentage of 88. Conidia in cortical extracts, on the other hand, germinated to not less than 98 % in every case.

Discussion

The examination of the seasonal dissemination of ascospores and conidia of *Nectria galligena* reveals that discharge is not limited to a relatively short seasonal period, such as has been demonstrated for the ascospores of the scab fungus *Venturia inaequalis* (Keitt & Jones, 1926). The discharge of *V. inaequalis* ascospores, under American conditions, can be predicted with fair accuracy, so that protective measures can be concentrated within the limited period of maximum effectiveness.

With *Nectria galligena*, however, ascospore discharge remains at a high level in all rainfall periods from November to April or May. During the summer, relatively few spores are present but, in the autumn, conidia are produced in large numbers while ascospore output continues to increase. The ascospores are, for the most part, forcibly expelled from the perithecia to a distance sufficient to expose them to the dispersing influence of air currents. Even if the mechanism for forcible discharge breaks down and the ascospores accumulate at the ostiole, these masses may yet become dispersed, as shown by their occasional appearance on the slides of the spore trapping experiments in the field. Ascospore discharge ceases when the perithecia are no longer saturated, but is not retarded by cold until the temperature falls below 5° C.

Germination of the spores occurs under favourable conditions in a few hours and will take place over a temperature range of 2-30° C. Growth of the vegetative mycelium also takes place slowly at 2° C. in culture. A fungus able to maintain spore discharge and growth at relatively low temperatures, and to continue production of spores throughout the autumn, winter and spring has clearly unusual potentialities for infection of the dormant tree.

It is generally recognized that canker of apples is of much greater importance in the west than in the east of England. It is probable that this is due to climatic factors. The present investigation has emphasized that rainfall is necessary for the dissemination of the spores of *N. galligena*. It is also probable that the observation, due to Zeller (1926), that dry weather delays formation and maturing of the perithecia, will also be applicable in the east of England.

The very large numbers of ascospores collected on the vaselined slides make clear the potential infective power of cankered wood in relation to adjacent shoots and to neighbouring trees. The prompt removal of all such sources of infection is therefore of great importance in controlling the disease.

SUMMARY

1. The fungus causing apple canker is described and its identity with *Nectria galligena* Bres. is confirmed.

2. The methods of dispersal of the spores of *N. galligena* have been examined. The ascospores are discharged forcibly from the perithecium, the eight spores of each ascus typically being ejected simultaneously.

3. Field investigations have shown that spores are discharged during wet periods at all times of the year. Ascospore discharge reaches its maximum in January–February and falls to a minimum in the summer months.

4. The dispersal of conidia occurs during wet periods in the spring and autumn.

5. Germination both of ascospores and conidia occurs over a range of temperature from 2 to 30° C., the optimum being at 20° C.

The author wishes to take this opportunity of recording his indebtedness to Mr R. W. Marsh who suggested this problem, and who throughout its progress has always been ready with helpful advice and criticism.

REFERENCES

ALLESCHER, A. (1892). In *Ber. bot. Ver. Landshut*, **12**, 130.

APPEL, O. & WOLLENWEBER, H. W. (1910). Grundlagen einer Monographie der Gattung *Fusarium*. *Arb. biol. Abt.* (*Anst.-Reichsanst.*), *Berl.*, **8**.

BRESADOLA, J. (1901). In Strasser, *Pilzfl. Sonntagbl.* **4**, 413.

CAYLEY, D. M. (1921). Some observations on the life history of *Nectria galligena* Bres. *Ann. Bot., Lond.*, **35**, 79.

DICKSON, B. T. (1925). *Diseases of Fruit Crops in Quebec: Apple Diseases.* Macdonald College, Quebec.

DILLON-WESTON, W. A. R. (1927). Notes on the canker fungus. *Trans. Brit. mycol. Soc.* **12**, 5.

GOETHE, R. (1904). *Über den Krebs der Obstbäume.* Berlin.

HARTIG, R. (1880). Der Krebs der Laubholzbäume. *Untersuch. forstbot. München*, **1**, 209.

VON HÖHNEL, F. & WEESE, J. (1910). Zur Synonymie in der Gattung *Nectria*. *Ann. mycol., Berl.*, **8**, 464.

KEITT, G. W. & JONES, L. K. (1926). Studies of the epidemiology and control of Apple Scab. *Res. Bull. Wis. agric. Exp. Sta.* no. 73.

MOORE, M. H. (1933). Some field observations on Apple Canker. *Ann. Rep. East Malling Res. Sta.* 1933, p. 166.

MORITZ, O. (1930). Studien über Nectriakrebs. *Z. PflKrankh.* **40**, 251.

OGILVIE, L. (1935). The fungus flora of apple twigs and branches and its relation to apple fruit spots. *J. Pomol.* **13**, 140.

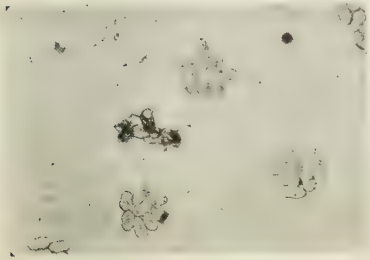


Fig. 1.

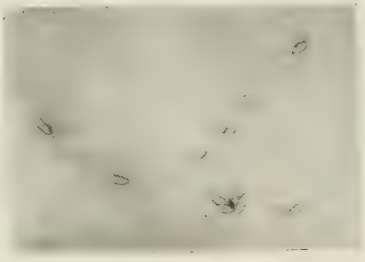


Fig. 2.

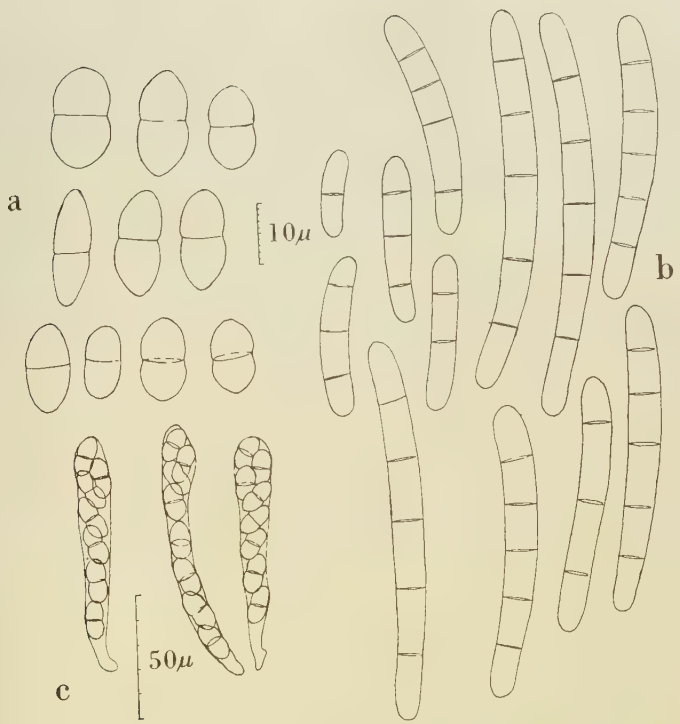


Fig. 3.

OSTERWALDER, A. (1931). Die *Nectria*-Kelchfäule an Äpfeln. *Gartenbauwiss.* **5**, 469.
RICHTER, H. (1929). Die wichtigsten holzbewohnenden Nectrien aus der Gruppe der Krebserreger. *Z. Parasitenk.* **1**, 24.
TULASNE (1865). *Selecta fungorum carpologia*, **3**, 73. Paris.
WEESE, J. (1911). Zur Kenntniss des Erregers der Krebskrankheit. *Z. landw. Vchsw. Öst.* 1911, p. 872.
WESTERDIJK, J. & VAN LUIJK, A. (1924). Untersuchungen über *Nectria coccinea* und *N. galligena*. *Meded. phytopath. Lab. Scholten*, **6**, 3.
WILLKOMM, M. (1866). *Die microscopischen Feinde des Waldes*, p. 103. Dresden.
WILTSHIRE, S. P. (1914). A note on the relation between Woolly Aphis and canker. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1914, p. 94.
—— (1919). The Apple Canker fungus. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1919, p. 23.
—— (1921). Studies on the Apple Canker fungus. I. Leaf-scar infection. *Ann. appl. Biol.* **8**, 182.
WOLLENWEBER, H. W. (1913*a*). Studies on the *Fusarium* problem. *Phytopathology*, **3**, 24.
—— (1913*b*). Abgrenzung von Pilzgruppen. *Phytopathology*, **3**, 197.
—— (1929). Über Fruchtformen der krebserregenden Nectriaceen. *Z. Parasitenk.* **1**, 138.
ZELLER, S. M. (1926). European canker of pomaceous fruit trees. *Bull. Oregon agric. Exp. Sta.* no. 222.

EXPLANATION OF PLATE XXIII

Figs. 1 and 2. Groups of *N. galligena* ascospores caught on vaselined slides in the field. Fig. 1 × 232. Fig. 2 × 182.

Fig. 3. Spore stages of *N. galligena*: *a* ascospores, *b* conidia, *c* asci.

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OBSERVATIONS ON APPLE CANKER

II. EXPERIMENTS ON THE INCIDENCE AND CONTROL OF SHOOT INFECTIONS[1]

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(With 2 Text-figures)

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I. Introductory

The records given by Munson (1939) show that inoculum of *Nectria galligena* is present in the field throughout the year, being specially abundant during the winter and spring. Any spraying programme for protecting apple shoots from canker infection is therefore likely to be difficult and costly and, in dealing with mature trees, it is probable that the principal method of combating canker will continue to be by cultural treatments. The treatment generally recommended is grassing down, designed to check susceptibility to canker by reducing excessive vigour. Nursery material and other young trees, however, present a special problem in that strong growth is highly desirable in the early years of tree development. Further, young apple trees are readily accessible and valuable in relation to their size; consequently they may merit special

[1] Read before the Association on 9 December 1938.

control treatments which would not be justifiable on older trees. For these reasons, this series of canker investigations has been started by a consideration of infections on trees not more than five years planted.

The researches of Wiltshire (1914, 1921, 1922) revealed the morphological details of canker infection of shoots through leaf scars, apple-scab lesions and woolly-aphis galls. Infections may also occur through major bark disruptions such as those due to mechanical injury or pruning cuts. Since in young trees the majority of cankers arise around leaf scars and pruning cuts, infections through these two channels were chosen for special study.

II. Inoculation experiments

(1) *On leaf scars*

Wiltshire (1919) and Wiltshire & Spinks (1920) record that in a batch of trees obtained by crossing the two cider varieties Medaille d'Or and Kingston Black the leaf-scar infections were not seen before the spring. However, in the varieties King of the Pippins and Quarrenden, leaf-scar infections of the 1920 wood were found as early as 23 September 1920. In the present investigation the attempt was made to determine, for the variety Cox's Orange Pippin, the variation in susceptibility to leaf-scar infection throughout the dormant season.

The material utilized comprised forty 2-year-old trees of Cox's Orange Pippin potted in December 1936 and kept out of doors except during freezing weather when they were moved into a cool greenhouse. All the trees were transplanted into open ground on 18 March 1938. Six series of experiments were carried out on the following dates: 26 November 1936, 29 January 1937, 5 April 1937, 4 October 1937, 5 January 1938 and 14 March 1938. For each series of experiments forty 1-year shoots were selected of which half were sprayed as described in a later section.

The twenty sprayed shoots, together with the corresponding twenty unsprayed shoots, in each series, were inoculated, without wounding, by placing a fragment of agar bearing mycelium of *N. galligena* on one of the leaf scars, the inoculation being then covered with a crêpe rubber bandage. In the 1936–7 experiments all inoculations were made on the same day as the sprayings, but in the 1937–8 series, half of the inoculations were made on the day of spraying and half a week later. The crêpe rubber bandages were removed from the inoculations after a fortnight and the shoots were examined for canker infections at frequent intervals. The results of the inoculations are given in Table II.

It will be noted that infection in the winter months was practically nil under the conditions of this experiment. In October 1937, six trees out of the twenty unsprayed became infected and, in April 1937, sixteen out of the twenty. Trees inoculated in the spring of 1938, however, did

not become infected, but at that time the trees received a serious check through being transplanted on 18 March during a period of frost and drought which continued until the end of April. Previous observations have suggested that trees thus checked are highly resistant to canker infection.

(2) *On pruning cuts*

Cankers arising at pruning cuts are commonly noted in the field in the spring and early summer, but no records have been found of experiments made in this country to determine the time at which infections of these pruning wounds originate. Zeller (1926) in Oregon noted that infections following pruning took place most readily in the autumn. It has been

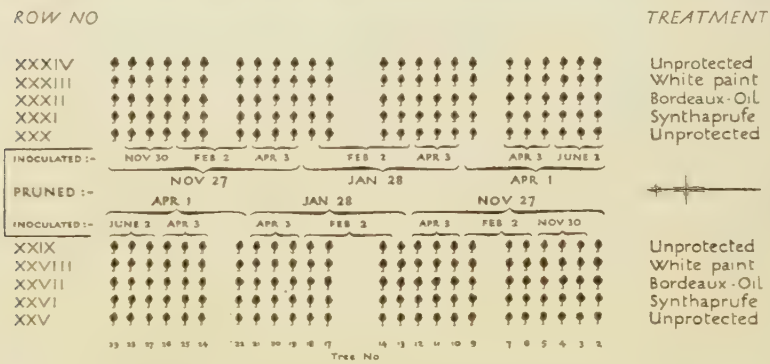

Fig. 1. Lay-out of wound protectant trial 1936–7.

shown by Munson (1939) that there is no periodic break in ascospore discharge from the autumn to the spring, so that the possibility of any variation in the susceptibility of the tree is of special interest. Further points chosen for investigation were the age of the wound in relation to resistance to infection and the merits of certain wound protectants.

The trees available for experiment comprised 240 Cox's Orange Pippins planted out in 1934–5 in ten rows of equal length (nos. XXV–XXXIV) running north and south (Fig. 1). To reduce the influence of tree position the treatments carried out on the western half of the block (rows XXV–XXIX) were repeated on the eastern half (rows XXX–XXXIV) in the reverse order. Experiments were carried out in each of the seasons 1935–6, 1936–7 and 1937–8. A typical lay-out is illustrated in Fig. 1, which gives a plan of the 1936–7 trial. It will be noted that 100 of the trees were pruned in November, seventy in January, and seventy in April, and that in each block ten trees remained uninoculated. The remainder of the November-pruned trees were inoculated, thirty within 1 week of pruning, thirty 2 months later and thirty 4 months

later. Similarly, the January-pruned and April-pruned trees were inoculated in two batches; one shortly after pruning, the other 2 months later.

The pruning treatment applied prior to 1937–8 was a shortening of a single leader on each tree, the terminal wound being used for treatment. In 1937–8, one secondary shoot on each tree was cut flush at its point of intersection with a 2-year-old leader, thus leaving a lateral wound. If the wounds were to be protected, the protectant material was applied immediately after wounding. The inoculations, made after the

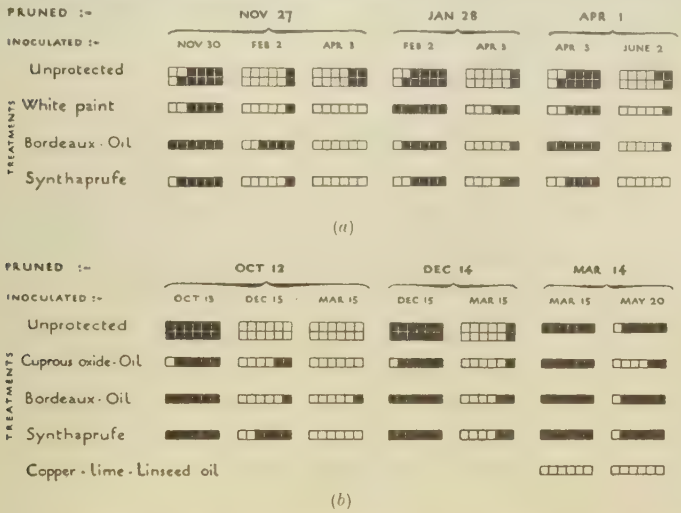

Fig. 2. (*a*) Results of wound protectant trial 1936–7. (*b*) Results of wound protectant trial 1937–8. Each square represents an inoculation: each black square represents a canker.

prescribed time, were carried out by placing fragments of an agar culture of *N. galligena* in contact with the protected or unprotected wound. The inoculation was then bound round with crêpe rubber which was removed a fortnight later. The 1935–6 season was used for a preliminary test of methods and materials: the results of the 1936–7 and 1937–8 trials are given in diagrammatic form in Fig. 2.

Considering first the unprotected pruning cuts, it is evident from these results that there was no period from October to March at which *N. galligena* mycelium failed to infect newly-made wounds. Infections initiated in December commonly required 6 months in which to become evident, but those starting in autumn or spring were plainly visible in 2 months. The age of the cut, i.e. the length of time elapsing between wounding and infection, was, however, of outstanding importance, and the great majority of infections arose from the inoculations made directly

after wounding. As the pruning cuts aged they acquired resistance to infection, and when the interval between wounding and inoculation was 2 months or longer, the proportion of successful infections was commonly low. An exception occurred in the pruning cuts of March 1938, which remained relatively susceptible in May of that year.

The effects of the wound protectant treatments will be considered in a later section, but it will be evident from Fig. 2 that, in general, the susceptibility or resistance of the cuts treated with these materials followed the same course as with the unprotected wounds.

III. Control measures

It has previously been noted that two important channels of canker infection are leaf scars and pruning cuts. As it is impracticable to treat leaf scars individually, they can be protected only by spraying the whole tree. The effectiveness of such protection will obviously be influenced by the wetting and spreading properties of the spray and by the tenacity of the fungicidal residue. Pruning cuts, however, may merit individual attention by the painting on of a protectant material, this application being restricted to the cut surfaces. The protectant paint may depend for its effectiveness either on toxicity to fungus spores, or on the presentation of a mechanical barrier, or on a combination of these factors.

The preparation of sprays and of paints which would be satisfactory in both fungicidal value and physical properties was made the subject of preliminary trials, followed by field experiments.

(1) *On leaf scars*

Laboratory tests. There have been numerous observations that spraying with lime sulphur does not directly control attacks by *N. galligena.*

In the present investigation, laboratory tests were carried out in which lime sulphur at a number of concentrations was atomized on to slides under standardized conditions to give a deposit of 0·008 ml. of spray/sq. cm. After being allowed to dry, the slides were sown with drops of a water suspension of ascospores of *N. galligena.* Comparable experiments were made with Bordeaux mixtures of different copper concentrations. The results are given in Table I, each figure being the mean percentage germination obtained by counting a total of 600 spores in six replicated drops.

Table I shows that even under conditions more favourable to the fungicide than those found in the field, lime sulphur was non-toxic to *N. galligena* spores at 1/11 strength and only partially toxic at 1/3·7. A mixture of 1 % lime sulphur with 3 % grade G petroleum oil was also found to be non-toxic. Sprays containing lime sulphur were therefore

Table I. *Toxicity of lime sulphur and of Bordeaux mixture to ascospores of* Nectria galligena

G. fungicide/l. of spray	% germination of *N. galligena* ascospores on slides sprayed with: Lime sulphur	Bordeaux mixture
0·11	—	56
0·33	—	43
1·0	—	35
1·1	97	—
3·0	—	17
3·3	97	—
9·0	—	5
10·0	93	—
30·0	96	—
90·0	98	—
270·0	42	—
810·0	0	—

omitted from further consideration, while Bordeaux mixture was chosen to form the fungicidal basis of a spray application.

Field trials. Wiltshire & Spinks (1920) record that spraying with copper stearate in December 1919 gave favourable results in controlling leaf-scar infections, but that a spray with 4–4–50 Bordeaux in November 1921 was unsuccessful. Moore (1933) states that Bordeaux spraying over a period of 5 years tended to reduce canker infection on Cox's Orange Pippin.

The prime requirements of a protectant spray for use on apple shoots are a high level of retention and of tenacity. It has been shown by Fajans & Martin (1938) that these requirements are best met by an oil-emulsifier-solid system with a minimum quantity of emulsifier. To provide a complete cover of the leaf scars on an apple shoot it is, however, necessary that the spray used should have adequate wetting and spreading power, which calls for an increase of the proportion of emulsifier in the oil-emulsifier-solid spray. After a number of preliminary tests on apple shoots the compromise reached in this investigation was a spray made up as follows:

Copper sulphate	8 lb.
Hydrated lime	16 lb.
Casein	4 lb.
Petroleum oil	1 gal.
Water	100 gal.

In this mixture the lime and the oil both provided tenacity, the casein conferred wetting power and the oil spreading power. Because of its wetting ability, the initial retention of the spray was relatively low.

In the present investigation spraying trials were carried out for 2 years in autumn, winter and spring on the trees of Cox's Orange Pippin previously described in the section on leaf-scar inoculation experiments. No infections, either on sprayed or unsprayed trees, resulted from the inoculations made in November 1936, January 1937 and January 1938. Of the twenty unsprayed trees inoculated in April 1937, sixteen trees became cankered, while in the corresponding sprayed series six showed

Table II. *Results of leaf-scar inoculation experiments*

No. of shoots	Date of spraying	Date of noculation	No. of infections
	1936	1936	
20	26 Nov.	26 Nov.	0
20	—	26 Nov.	0
	1937	1937	
20	29 Jan	29 Jan.	0
20	—	29 Jan.	0
20	5 Apr.	5 Apr.	6
20	—	5 Apr.	16
10	4 Oct.	4 Oct.	1
10	4 Oct.	11 Oct.	0
10	—	4 Oct.	1
10	—	11 Oct.	5
	1938	1938	
10	5 Jan.	5 Jan.	0
10	5 Jan.	12 Jan.	0
10	—	5 Jan.	0
10	—	12 Jan.	0
10	14 Mar.	14 Mar.	0
10	14 Mar.	21 Mar.	1
10	—	14 Mar.	0
10	—	21 Mar.	1

In addition, two uninoculated shoots developed cankers in spring 1937.

infection. In October of the same year, twenty unsprayed trees showed six infections as compared with one in the sprayed series. The full results are given in Table II.

(2) *On pruning cuts*

Materials. The practice of protecting pruning cuts has been much debated, and a summary of work on wound protectants is given by Howe (1915) who concludes that there is nothing to be gained by their use on apple wood. A recent paper by Moore (1933) records that uninoculated wounds on Cox's Orange Pippin wood remained free from canker whether treated with neat tar distillate, bituminous emulsion, colloidal sulphur, white lead paint or no protectant. Zeller (1926) recommends the treat-

ment of wounds by a paint made by mixing a proprietary copper-lime dust with linseed oil.

The list of materials tested in the present investigation is as follows:

A. Oil-Bordeaux made up of

copper sulphate	8 g.
hydrated lime	16 g.
casein	4 g.
petroleum oil (grade G)	10 ml.
water	1 l.

B. Cuprous oxide powder as specified by Horsfall (1938) in one-third its weight of edible cotton-seed oil.

C. Copper-lime-linseed oil consisting of

monohydrated copper sulphate ...	5 g.
hydrated lime	10 g.
boiled linseed oil	9 ml.

D. Commercial white lead paint[1] (white lead, conforming to B.S.S. no. 239, comprising 60 % of total solids).

E. "Synthaprufe" proprietary bituminous emulsion in liquid and paste forms.

F. Soft grafting wax (proprietary product).

G. Mercury-glycerine wound dressing (see Day, 1924) consisting of

mercuric cyanide	6·25 g.
mercuric chloride	6·25 g.
glycerine	3 l.
water	1 l.

In addition, tests were made of a number of Bordeaux pastes (copper sulphate 65 g., lime 130 g., water 1 l.) with and without cotton-seed oil and linseed oil.

Laboratory tests. Ascospores of *N. galligena* sown on slides painted with either Synthaprufe or white lead paint showed vigorous germination, the germ tubes growing freely alongside and through both paints. The oil-Bordeaux-casein mixture, in a comparable test, completely inhibited spore germination, but this material leaves a deposit which is too tenuous to be suitable for wound protection. Similarly, the mixtures of copper sulphate, lime, water and oils proved too free-flowing for field application. It appeared to be essential to add a dry material to the oil to obtain a paint of the required consistency, and a number of mixtures were made up similar to the paint recommended by Zeller (1926). The formula finally adopted is given above (C).

Further laboratory trials of the fungicidal value of certain of these materials were carried out by painting them on to the upper cut ends of

[1] Not the specially thickened paint recommended by Brooks & Moore (1925).

detached shoot portions, then placing *N. galligena* spores in a drop of water on the painted surface, the whole being enclosed in a moist chamber. After 24 hr. the spores were examined for germination. Of the materials mentioned above, only the mercury-glycerine dressing and the copper-lime linseed oil were entirely effective in preventing spore germination in these circumstances. The mercury dressing was found to be injurious to young shoots of apple and was therefore not employed in field trials.

Field trials. Field trials were carried out each winter from 1935 to 1938 on the 240 trees of Cox's Orange Pippin described in an earlier section. Certain materials which had not proved satisfactory in laboratory tests, but which were often applied in practice, were included for purposes of comparison. The complete list of materials tested on a field scale is as follows:

White lead paint; soft grafting wax; "Synthaprufe" bituminous emulsion; Bordeaux oil; cuprous oxide in cotton-seed oil; copper-lime-linseed oil. Full descriptions of these materials are given in a preceding section.

The technique of wounding has already been described, and a typical lay-out for the wound protectant trials is shown in Fig. 1. On all cuts to be protected, the protectant material was applied immediately after wounding. The inoculations were then made after the prescribed times by placing fragments of an agar culture of *N. galligena* on the wound and binding round with crêpe rubber which was removed a fortnight later.

It has previously been mentioned that the 1935–6 season was used for a preliminary test of materials and methods and, as one result of this trial, soft grafting wax was discarded as being too difficult to manipulate on small wounds. The results of the 1936–7 and 1937–8 experiments are presented in diagrammatic form in Fig. 2.

It must be emphasized that the wound protectants used were subjected to a very stringent test, a relatively large mass of fungus mycelium supplied with nutrient being held closely applied to the treated wound for a fortnight. In these circumstances, wounds treated with all but one of the materials tested became cankered as readily as the wounds left unprotected. The exception was the copper-lime-linseed oil mixture which, in a limited number of field tests, gave complete control in spite of the onerous conditions of the experiment.

Observations concerning the direct effect of the various wound protectants on the tissues of the apple shoots, confined to the variety Cox, showed that the cuprous oxide oil mixture caused local necrosis of the

bark and a marked browning of the wood. This discoloration, which was associated with the presence of gum in the xylem vessels, extended up to 4 in. from the painted surface. In the wounds treated with white paint; with Synthaprufe and with Bordeaux-oil there was delay in callusing and a shrinkage and cracking of the bark tissues surrounding the treat cut. The copper-lime-linseed oil appeared to have no adverse effect on callusing, and the cuts treated healed over as quickly as comparable non-infected and unpainted wounds.

IV. Discussion

It has been shown that lime-sulphur and tar-oil spraying have no effect on the production of *N. galligena* spores, while neither petroleum oil nor lime sulphur, at strengths used in the field, has any effect on their germination. The use of Bordeaux mixture on apples in this country has now been largely discontinued because of its tendency to cause fruit russeting when applied at the "pink" and "petal fall" stages. Unfortunately, this tendency is shown most seriously on the canker-susceptible dessert apples, Cox's Orange Pippin, James Grieve and Worcester Pearmain. The present-day routine spraying programme for apples—tar oil in December or January, petroleum oil in March, and lime sulphur in spring and summer—thus makes no direct contribution to the control of canker.

A consideration of the spore discharge records shows that the likelihood that shoot infections will be initiated during the summer months is low, so that spraying during this period will have only a minor effect on the incidence of canker. Again, the inoculation experiments on leaf scars indicate that unwounded shoots do not readily become infected during December or January. For this reason the addition of fungicides to the tar-oil wash with the object of controlling canker does not seem likely to achieve success. The critical periods for leaf-scar infection are in the spring and autumn. In the present paper an instance is given of the beneficial effect of a Bordeaux-casein-oil spray, applied in April, in reducing canker infection. Such a spray could be introduced into the routine programme by adding Bordeaux mixture to the petroleum oil emulsion now applied in March. Alternatively, Bordeaux mixture could be substituted for lime sulphur in the first pre-blossom or "green flower" scab spray. However, the combined Bordeaux-petroleum spray would be preferable, since the presence of the oil would improve wetting and sticking properties.

In the autumn, the protection of trees against canker infections of

leaf scars could not be achieved without including a spray application outside the present routine programme. A spray with Bordeaux mixture, preferably mixed with an oil emulsion, merits consideration for nursery stock and young trees of canker-susceptible varieties. It should be applied as soon as possible after leaf fall.

Turning to the problem of pruning-cut infections, it is noteworthy that dry and cold conditions are unfavourable to the discharge and germination of the spores of *N. galligena*, thus suggesting an advantage in choosing spells of dry frosty weather for pruning operations. Another factor concerning time of pruning is the prolonged susceptibility of wounds made in March. This phenomenon, taken together with the rising spring temperatures favouring spore germination, indicates that late pruning is prejudicial to canker control.

The behaviour of the pruning cuts made in March forms an exception to the general rule that wounds 2 months old or more show a natural resistance to infection. This feature of the results dealing with the inoculation and protection of pruning cuts necessitated a new approach to the problem of wound treatment. Much previous work on this subject has been directed to the provision of a weatherproof seal for large wounds which would give mechanical protection for a long period—possibly several years. Sealing materials of low fungicidal value, however, proved ineffective against inoculations with *N. galligena*, and numerous observations have shown that they are not highly effective against canker in general orchard practice. For a pruning cut of relatively limited size, made during the winter months, the requirement for canker control is a covering which will prevent disruption of the surface and maintain an effective concentration of fungicide on the wound for the period before natural resistance is established. The mixture of monohydrated copper sulphate with hydrated lime, compounded to a thick paint with linseed oil, shows promise of fulfilling this requirement without causing injury to the shoots or interfering with callusing, but large-scale trials with the material are necessary before its value can be established.

V. Summary

1. Inoculations with *N. galligena* on unwounded shoots of apple show that canker infections through leaf scars may be initiated in October and April but not in November and January. Shoots sprayed before inoculation with a Bordeaux-casein-oil mixture gave a much lower proportion of infection than those unsprayed.

2. Inoculations with *N. galligena* on pruning cuts showed that freshly made pruning cuts on apple shoots are susceptible to canker infection throughout the dormant season. Pruning cuts made during autumn and winter acquire a natural immunity to infection within 2 months.

3. Several wound sealing materials applied to pruning cuts failed to give protection from canker when the treated surfaces were inoculated with *N. galligena*. The most promising wound protectant tested was a mixture of monohydrated copper sulphate, hydrated lime and linseed oil.

The author wishes to record with appreciation the help given by Mr R. G. Munson who carried out the major portion of the field work described in this paper. Thanks are also due to Mr E. H. Wilkinson for assistance in inoculating and recording.

REFERENCES

Brooks, F. T. & Moore, W. C. (1925). Silver-leaf disease. V. *J. Pomol.* **5**, 61–97.

Day, L. H. (1924). Experiments in control of cankers of fire blight. *Phytopathology*, **14**, 478–80.

Fajans, E. & Martin, H. (1938). The incorporation of direct with protective insecticides and fungicides. III. Factors affecting the retention and spray residue of emulsions and combined emulsion-suspensions. *J. Pomol.* **16**, 14–38.

Horsfall, J. G. (1938). Combating damping-off. *Bull. N.Y.* (*Geneva*) *agric. Exp. Sta.* no. 683.

Howe, G. H. (1915). The effect of various dressings on pruning wounds of fruit trees. *Bull. N.Y.* (*Geneva*) *agric. Exp. Sta.* no. 396.

Moore, M. H. (1933). Some field observations on apple canker. *Ann. Rep. East Malling Res. Sta.* 1933, pp. 166–75.

Munson, R. G. (1939). Observations on apple canker. I. The discharge and germination of ascospores of *Nectria galligena*. *Ann. appl. Biol.* **26**, 440–457.

Wiltshire, S. P. (1914). A note on the relation between woolly aphis and canker. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1914, p. 94.

—— (1919). The apple canker fungus. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1919, pp. 23–9.

—— (1921). Studies on the apple canker fungus. I. Leaf-scar infection. *Ann. appl. Biol.* **8**, 182–92.

—— (1922). Studies on the apple canker fungus. II. Canker infection of apple trees through scab wounds. *Ann. appl. Biol.* **9**, 275–81.

Wiltshire, S. P. & Spinks, G. T. (1920). Apple tree canker. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1920, pp. 82–3.

Zeller, S. M. (1926). European canker of pomaceous fruit trees. *Bull. Oregon agric. Exp. Sta.* no. 222.

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PHYTOPHTHORA ERYTHROSEPTICA PETHYBR. IN RELATION TO ITS ENVIRONMENT

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(With 2 Text-figures)

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Introduction

In 1929 the writers commenced a study of pink rot of the potato and, later, published their observations on the disease (Cairns & Muskett, 1933*a*). The present account describes an investigation made with the object of obtaining a clearer conception of the role played by the more salient environmental factors in the physiology of the normal pathogene, *Phytophthora erythroseptica* Pethybr., and in the incidence of the disease caused by this organism.

Environmental factors

Staling

It has been shown by Brown (1925) and others that, if a fungus is grown in a medium where its staling products tend to accumulate, the resultant staling effect on the growth of the organism may be pronounced. In view of this, observations were made on the staling effect by (*a*) plotting a periodic growth rate, and (*b*) comparing the rates of growth in different depths of the same medium.

The daily growth rate of the fungus in plates of potato-mush and malt-extract agars at 22° C. was recorded by measuring the diameters of the colonies, the measurements being continued until the plates were fully covered (see Fig. 1). Comparison of the rates of growth in different depths of the same medium at 22° C. was made on oat and malt-extract agars, the measurements being taken when the fungus had grown almost to the edges of the plates (see Table I).

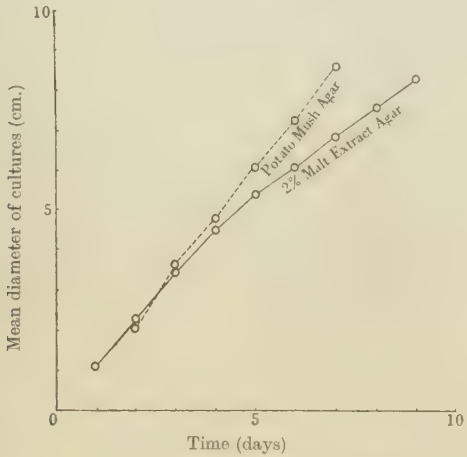

Fig. 1. Growth rates of *P. erythroseptica* at 22° C. on 2 % malt-extract agar and on potato-mush agar.

Table I

		Mean diam. (5 plates) cm.
Oat:	Plates containing 15 c.c. medium	8·2
	,, ,, 45 c.c. ,,	7·8
Malt:	,, ,, 10 c.c. ,,	7·8
	,, ,, 40 c.c. ,,	7·6

In all the *natural* media used in the present work the fungus grows, apparently at a constant rate, up to the edge of the plate, and it is evident that the staling effect is negligible on these media. No allowance was therefore made for staling in the subsequent work where these media were employed.

Nevertheless, a constant growth rate does not occur in all media; it has been noted that the rate of growth decreases with age and ceases about 1 cm. from the edge of the plate in cultures on certain synthetic media and notably where potassium nitrate is used as a source of nitrogen.

Light

The following are details of an experiment on the effect of light on the growth and reproduction of *P. erythroseptica*.

Two groups, each of seven plates of malt-extract agar, were inoculated and incubated at laboratory temperature. The plates of one group (*a*) were placed on a white surface near a large window, so that they might be exposed to daylight, while at the same time they were further exposed to the continuous light of a 100 W. lamp placed at a distance of 2 ft. above them. The other group (*b*) was enclosed in a well-aerated light-proof chamber, which consisted of a double box arrangement with two opposite air vents in each box, the vents of one box alternating with those of the other. Layers of black paper were loosely placed round the plates for further protection. The mean daily temperature was approximately 17° C. in group *a*, and 16° C. in group *b*, while the average growth measurements after 10 days were

Group *a*.	Exposed to continuous light (17° C.) ...	8·4 cm.
Group *b*.	Continuous darkness (16° C.)	7·7 cm.

These results were confirmed by a parallel series grown in potato-mush agar.

It thus appears that light has no appreciable effect on the growth rate of the fungus. The small difference in growth rate obtained may be explained by the temperature difference of 1° C. which occurred under the varying conditions.

Numerous oospores were produced under both conditions, and to apparently the same extent. They were particularly profuse in the potato-mush agar. No sporangia were found.

The effect of light on the production of sporangia in liquid media was not studied in detail, but on different occasions these were observed in cultures exposed to the light of the laboratory and also in those which had been kept in dark incubators. Waterhouse (1931) did not find light to be concerned in the production of sporangia in the several species of *Phytophthora* which she studied.

Acidity

In a provisional investigation of the relationship of *P. erythroseptica* to this factor, two media—2 % malt-extract agar and 15 % potato-mush agar (raw tuber 150 g./l.)—were prepared. Each medium was then divided into parts and sterilized, after which the reaction was adjusted in each part to give series ranging by steps of 0·2–1·0 from *p*H 2·5 to 11·0. Each medium was poured immediately without further sterilization. No supplement of a buffer substance was given. On cooling, the plates were inoculated from cultures grown on media adjusted to a corresponding reaction. They were then incubated at 18° C.

The results showed that the acid limit for growth was *p*H 3·2, and that the optimum covered a wide range in the region *p*H 6·0–7·0, but because

of the acid drift the alkaline limit could not be ascertained. For example, a malt medium of *p*H 10·0, in which the fungus had become established and grown well, had dropped to *p*H 8·4 during the experimental period.

Oospores were produced freely over the greater part of the *p*H growth range but not towards the limits of acidity or alkalinity. Sporangia were not observed on any of these gel media.

Like staling and light it was apparent that this factor need not be taken into account in the experiments dealing with moisture and temperature.

Moisture

Reproduction. Pethybridge (1913) found that sexual reproduction in *P. erythroseptica* occurs freely in gel- and liquid-media, but asexual reproduction is limited to liquid. This has been confirmed in the present work. Both Pethybridge (1913) and Murphy (1918) observed sporangia developed occasionally in drops of condensed moisture on aerial hyphae, but this has never been the experience of the writers. More recently Leonian (1934) has shown how asexual reproduction may be readily induced by aquatic conditions following culture of the organism in a highly nutrient pea medium; and McKay (1937) finds that older cultures (6–12 months) produce sporangia more readily than younger cultures (1 month).

Infection. Although typical pink rot of the potato is normally caused by *P. erythroseptica*, or more rarely by *P. megasperma* (Cairns & Muskett, 1933*b*), a somewhat similar condition of the tuber may result from simple asphyxiation such as would be caused by the flooding of land carrying a potato crop. This is assumed by many farmers who commonly refer to the disease as "Waterslain" or "Drowning". Work carried out under aseptic conditions in this laboratory has shown that the death of the tuber, or the lowering of its resistance to such an extent that it becomes readily invaded by putrefactive organisms, occurs after submergence in water for a period of at least 24 hr. (temp. 16–20° C.). The decline of the tuber by such asphyxiation is accompanied by symptoms, which although somewhat atypical, bear a close resemblance to those of pink rot resulting from parasitic causes. In so far as *P. erythroseptica* is concerned in the causation of pink rot, excessive soil moisture is also conducive to the incidence of the disease, in which case the infection of the tuber is frequently *not* via the mother stolon.

The writers have frequently observed in cases where tubers in storage have contracted the disease and where infection has occurred after their detachment from the mother plant, that the infection often occurs at the

"rose" end of the tuber. They have further shown by experiment that tubers may become directly affected with the disease when placed in moist soil artificially contaminated with the parasite. The general results of an experiment of this nature which has already been described (Cairns & Muskett, 1933*a*) are as follows:

(1) Of 241 tubers stored in moist soil contaminated with *P. erythroseptica*, eighty-eight became infected with pink rot.

(2) Fifty-six of these eighty-eight tubers, where they were only partly affected, showed initial attack at the "rose" end, the diseased area usually radiating from an "eye". The soil in this experiment was of approximately the maximum moistness provided by capillarity from basal pans, and the temperature was that of January–February out-of-doors.

In order to obtain more information regarding the conditions governing direct infection of the tuber, the following experiment was undertaken.

A number of healthy potato tubers were washed and kept in a moist atmosphere for about 4 days in order to allow the healing of any wounds caused by washing. A glass ring, approximately 2·0 cm. in diameter and 1·0 cm. deep, was then fitted to each of the tubers by means of sealing cement and paraffin wax. The tubers were divided into two lots which were ringed as follows: (*a*) with the ring enclosing an "eye", (*b*) with the ring fitted on a portion of the tuber not involving an "eye". After ringing, the tubers were left for a further 2 days when the rings were filled with a water extract from the soil used in the previous experiment, and in each was placed a small inoculum (5–6 sq. mm.) of a 5–6 days old culture of *P. erythroseptica*. The tubers were then put in glass potato dishes and placed in an incubator at 22° C. The results obtained from this experiment were:

Variety	Locus of ring	No. of tubers	No. of tubers infected
Arran Victory	Internode	10	0
	"Eye"	7	6
Kerr's Pink	Internode	8	0
	"Eye"	11	7

With each of the above four lots a further two tubers were similarly treated but no *P. erythroseptica* was added. All of these control tubers remained healthy throughout the experiment.

The above results confirm those obtained by the preliminary trials and show that under conditions providing ample moisture, the healthy tuber readily contracts infection through the "eye". That such conditions are necessary is confirmed by the fact that in two further experiments where the inoculum was placed directly on the "eye" or the internodal region of the tuber without the use of a ring containing free water, no infection was obtained. Although the actual mode of infection was not observed, it was seen that sporangia were produced by the inoculum after its inclusion in the fluid within the ring.

Temperature

The effect of temperature has been investigated in relation to the growth and reproduction of the fungus and its infection of the potato tuber.

Growth. The relation of temperature to growth rate has been studied on oat-, potato- and malt-extract agars. The results obtained are presented in Fig. 2, where it will be seen that the temperatures for the

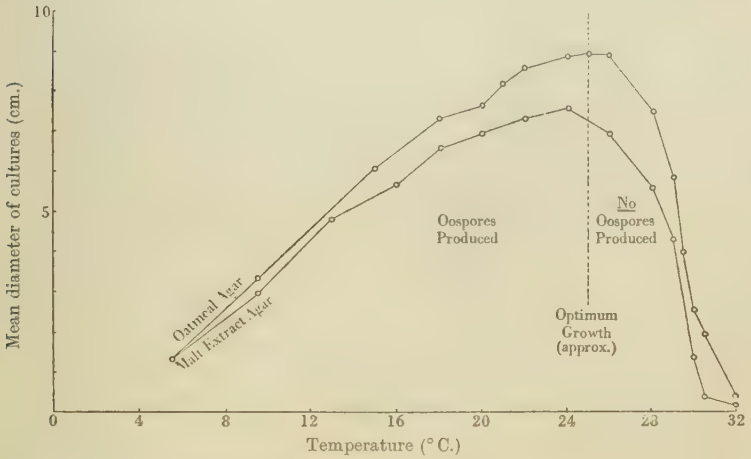

Fig. 2. Temperature-growth relationship for *P. erythroseptica* when growing on oatmeal agar and malt-extract agar.

minimum, optimum and maximum growth rates approximate 5, 25 and 31° C. respectively.

Infection. It has been observed by farmers that the incidence of pink rot in stored potatoes increases as spring succeeds winter, i.e. with a rise in the mean daily temperature. The following experiments were made to determine the effect of temperature upon direct infection of the potato tuber.

Five groups, each of twelve tubers (Arran Victory), were fitted with glass rings as previously described. In ten tubers of each group the ring was fitted so as to include an "eye", while in the remaining two it was fitted to the internodal region. The rings were filled with sterilized bog soil (peat) extract, and inoculations were made from a young culture of *P. erythroseptica* on oat agar. The tubers were then placed in glass potato dishes lined with moist absorbent paper, and each of the five groups incubated under different conditions of temperature. When infection had taken place and the

disease had progressed so far in a tuber that it could be identified by external symptoms, the tuber was removed and cut in half through the point of inoculation in order to obtain the confirmatory colour symptoms. The experiment (*a*) was allowed a duration of 21 days and was repeated (*b*) with a duration of 28 days. Details and results are given in Table II.

At the end of the experimental period the tubers of group A were transferred to a temperature of 20° C. for 14 days, when three tubers in A (*a*) and four tubers in A (*b*) had become affected with the disease through "eye" inoculation. Group E (*a*) was similarly treated, with the result that nine of the tubers inoculated at an "eye" contracted the disease.

Table II

Group	Storage conditions and temp. (° C.)		No. of infected tubers: "Eye" inoculation	No. of infected tubers: Internode inoculation	Time taken for disease to become obvious days
A	Refrigerator	(*a*) 5–7	0	0	—
		(*b*) 7–9	0	0	—
B	Laboratory	(*a*) 12–15	8	0	20
		(*b*) 17–20	9	0	16
C	Incubator	(*a*) 19–20	7	0	13
		(*b*) 20	6	0	7
D	Incubator	(*a*) 23–25	8	0	9
		(*b*) 24–25	9	0	10
E	Incubator	(*a*) 27–29	1	0	—
		(*b*) 30	0	0	—

The results of these experiments provide ample evidence of the relation between temperature and direct infection of the tuber. It would appear that below 10° C. and above 30° C. infection rarely takes place, and that in the intervening temperature range optimum conditions for infection and development of the disease occur in the neighbourhood of 20–25° C.

Discussion

In any discussion of pink rot of the potato it must be borne in mind that the disease may be caused by organisms other than *P. erythroseptica*, and that rather similar symptoms may be produced in tubers the tissues of which have been killed through exposure to lethal substances or through prolonged submergence in water or waterlogged soil. In many cases, however, apart from the characteristic coloration, the symptoms are somewhat atypical where *P. erythroseptica* is not the pathogene. The present investigation, which is concerned solely with the normal pink rot pathogene, *P. erythroseptica*, has elucidated considerably the relationship of this organism to its environment, and affords a fuller conception of the mode of tuber infection in the course of farm practice.

Although in artificial media in Petri dishes there was no appreciable

decrease in the rate of growth of the fungus through accumulation of its *own* staling products, this may not be true in the soil where the antagonistic effect of other organisms may be very pronounced. It was found that, when growing in artificial media, the fungus was intensely intolerant towards contaminants in general.

Under soil conditions light would not be involved except in the surface layers and, when investigated experimentally, this factor produced no significant effect.

Modification of the relative acidity of artificial media showed that the limits for growth were *p*H 3·2 and *circa* *p*H 9·0–10·0, with an optimum in the region of *p*H 6·0–7·0. Sexual reproduction was confined to within the limits *p*H 4·0–8·0, with an optimum at *p*H 6·0–7·0; outside this range oospores were not found, even in 6-week-old cultures. Asexual reproduction was not induced by any *p*H in a gel medium, but this effect was not studied in liquid media in detail. In view of this tolerance of *P. erythroseptica* to a wide range of hydrogen-ion concentration, and if it be considered that the soil in the potato-growing lands of Northern Ireland has a general reaction of *p*H 5·0–6·5, and that the most favourable soil reaction for growth of the potato is within the limits *p*H 4·8–5·7 (Comber, 1927), then it is obvious that any modification of soil acidity to inhibit development of *P. erythroseptica* is impracticable.

In this connexion the addition of lime and acid to contaminated land on two occasions did not offer any conclusive evidence as to decrease or increase in disease intensity. Nevertheless, lime as a soil flocculant should assist in improving drainage and thus tend to limit the incidence of the disease.

Sporangia were only produced under aquatic conditions, an observation which may be correlated with the fact that moisture is of primary importance in cases of *direct* infection of the potato tuber such as occur in the pit or clamp. The experiments dealing with moisture and infection generally indicated this, particularly where rings containing soil solution were fitted on different *loci* of the tuber. These experiments also demonstrated that *direct* infection is normally via the "eye" of the tuber. Before digging, infection is normally via the stolon and is therefore *indirect*. In laboratory experiments tubers in contaminated soil remained free from infection when its moisture content was not higher than about 19 %, but where the moisture content was raised to 24 %, infection readily occurred. With soil at its maximum moisture content all tubers became readily affected with pink rot, but attack by *P. erythroseptica* under these conditions tended to become obscured by the

possibility of asphyxiation of the tubers and attack by putrefactive organisms. Where severe outbreaks of pink rot have been reported from farms it has been observed that the soil had been abnormally wet for a period of several days previous to the outbreak. On the other hand, it has been observed that, in land known to be contaminated with *P. erythroseptica*, the incidence of pink rot was negligible in a dry season. In a field test, in light, well-drained soil, fifty tubers were planted in ridges with a plate culture of *P. erythroseptica* and portions of pink rot tubers placed in close proximity to each; yet all the tubers gave rise to healthy plants and there was no pink rot in the crop, which was similar in quantity to that of the uncontaminated (control) plot.

The results of an investigation into the temperature relationships of *P. erythroseptica* demonstrated one point of particular interest, i.e. that sexual reproduction only occurs between the minimum and optimum temperatures for growth (5–25° C.). The effect of temperature on the production of sporangia was not examined critically; but infection of tubers was inhibited above 25° C.—an effect which seems to be related to the production of sporangia, assuming that zoospores are the agents of infection under such conditions. Normal summer soil temperatures of about 20° C. would be very favourable for the growth of, and infection by, the parasite.

Any correlation of soil temperatures with the temperature relationships of *P. erythroseptica* must be very general, yet there is obviously a definite relation between them. Taking as typical the monthly *average* temperatures for a soil depth of 1 ft. at Hillsborough, Northern Ireland, it is seen that over a period of 5 years (1930–5) these averaged during the potato-growing season: April 7·3° C., May 10·4° C., June 13·7° C., July 15·5° C., Aug. 15·1° C., Sept. 13·4° C. and Oct. 10·6° C. Potatoes are grown at a depth of much less than 1 ft. and are therefore subject in summer to an average temperature slightly higher than that recorded, coupled with a correspondingly greater diurnal variation. The curves of the Oxford values given by Keen & Russell (1921) for depths of 6 and 12 in. are similar, the temperature at the shallower depth being less than 1° C. higher in summer and 1° C. lower in winter. At the temperatures recorded at Hillsborough for the dead season—Nov. 7·6° C., Dec. 6·0° C., Jan. 5·1° C., Feb. 4·8° C. and March 5·1° C.—the growth of *P. erythroseptica* must be practically inhibited, the more so since the figures for the cultivated layer would be less than those given, which are for 12 in.

In general, therefore, with *P. erythroseptica* present in soil otherwise favourable to the fungus, it would appear that warm, moist weather

persisting for a period of several days is most likely to encourage the onset of pink rot in the potato crop. That this is so has been observed by the writers on several occasions, and it was particularly noticeable during 1936 when, during an abnormally wet July and August, vigorous crops became affected with pink rot within a short time. These observations on temperature and moisture are significant in relation to the statement made by Pethybridge (1913) to the effect that the disease is of much more importance in the west of Ireland.

Summary

1. The present contribution, as part of a general study of pink rot of the potato, deals with *Phytophthora erythroseptica* Pethybr. in its relation to staling, light, acidity, moisture and temperature.

2. In natural media the fungus is not, or but very slightly, affected by its own staling products, but, in general, is intensely intolerant to the presence of other fungi and bacteria.

3. Ordinary daylight is not concerned in the growth or reproduction of the fungus.

4. Growth occurs over a wide *p*H range (3·2 to *circa* 9·0–10·0); sexual reproduction occurs over this range except towards the limits of acidity and alkalinity; asexual reproduction was not investigated experimentally. It is considered that modification of the soil reaction, within practical limits, does not offer a means for control of the disease.

5. The moisture factor controls directly the incidence of the disease in contaminated soil—with *low* moisture infection is inhibited; with *normal* moisture infection occurs via the dead or moribund stolon through the "heel" of the tuber; with high moisture the tuber is infected directly in the region of an "eye". Sporangia are only formed under aquatic conditions and it is considered probable that "eye" infection is brought about through the agency of zoospores.

6. The temperature limits of growth are 0–32° C. with an optimum at 25° C. Oospore production occurs only between the optimum and minimum growth temperatures (0–25° C.), being inhibited above the growth optimum. Asexual reproduction seems to be similarly related to temperature.

The writers wish to acknowledge their indebtedness to Mr R. W. Hale for placing at their disposal the data regarding soil temperatures recorded at the Agricultural Research Institute, Hillsborough.

REFERENCES

Brown, W. (1925). Studies in the genus *Fusarium*. II. *Ann. Bot., Lond.*, **39**, 373.

Cairns, H. & Muskett, A. E. (1933*a*). Pink rot of the potato. *Ann. appl. Biol.* **20**, 381.

—— —— (1933*b*). *Phytophthora megasperma* causing pink rot of the potato. *Nature, Lond.*, **131**, 277.

Comber, N. M. (1927). *An Introduction to the Scientific Study of the Soil*, p. 105. Edward Arnold and Co.

Keen, B. A. & Russell, E. J. (1921). The factors determining soil temperature. *J. agric. Sci.* **11**, 211.

Leonian, L. H. (1934). Identification of *Phytophthora* species. *Bull. W. Va. agric. Exp. Sta.* no. 262.

McKay, R. (1937). Potassium permanganate as an aid to the production of asexual fructification by *P. erythroseptica* Pethybr. *Nature, Lond.*, **139**, 802.

Murphy, P. A. (1918). The morphology and cytology of the sexual organs of *Phytophthora erythroseptica*. *Ann. Bot., Lond.*, **32**, 115.

Pethybridge, G. H. (1913). On the rotting of potato tubers by a new species of *Phytophthora* having a method of sexual reproduction hitherto undescribed. *Sci. Proc. R. Dublin Soc.* **13** (N.S.), 529.

Waterhouse, G. M. (1931). The production of zoonidia in the genus *Phytophthora*. *Trans. Brit. mycol. Soc.* **15**, 311.

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SKIN SPOT (*OOSPORA PUSTULANS* OWEN & WAKEF.) OF THE POTATO, AND ITS CONTROL BY TUBER DISINFECTION

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(With Plate XXIV and 1 Text-figure)

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I. Introduction

Skin spot (*Oospora pustulans* Owen & Wakef.) is a disease which is not generally recognized by the farmer as causing damage to the potato tuber. As the lesions are superficial and do not extend to any great depth in the flesh, the loss in ware tubers with few exceptions is practically negligible. With seed potatoes the disease is of some consequence, as severe attacks, especially in the region of the eyes, may prevent sprouting and thus cause "misses" in the crops. Recent records in Great Britain (Ministry of Agriculture, etc. 1937), where losses of from 20 to 30 % in stocks of seed have been noted, would suggest that the disease is of some importance. The nature of the occurrence of skin spot is also deceptive. A supply of seed purchased or selected in the autumn or early winter may confidently be stored as perfectly sound and apparently free from the disease. When examined in the spring, however, it may be discovered that the disease has developed during storage and that many of the tubers have failed to sprout. Such an occurrence may result in the planting of a smaller acreage than originally intended since, at that time of the year, it is often impossible to obtain a further supply of suitable seed. The planting of diseased seed may also result in "misses" in the crop.

The methods generally recommended for the prevention of losses due to skin spot are usually of a precautionary nature. The selection and

boxing of seed immediately after lifting, in conjunction with efficient and hygienic storage, will do much to reduce the incidence of the disease, while the rejection of non-sprouted or poorly sprouted tubers at planting time assists in preventing "misses" in the field. It was thought that a more direct method of control which would prevent the development of the disease should prove to be of considerable value, and it was with this aim that experimental work was commenced in 1933 to investigate the effect of tuber disinfection.

II. Preliminary experiments

Experiments were made during 1933–5 to test the value of the disinfection of seed potatoes before planting for preventing the development of skin spot in the resultant crop during storage. The seed planted in all these experiments was selected as severely affected with skin spot, and was disinfected a few weeks before planting. Disinfection was carried out by immersing the tubers in solutions of various materials, the immersion period varying from short or "instantaneous" dips (0·5–1 min.) to steeps of 90 min. duration. In addition to mercuric chloride and formalin, two proprietary organo-mercury preparations A and B were employed. The potatoes were planted in land which had not previously borne a potato crop and the plots were randomized, each plot consisting of one drill 20 yd. in length. In one experiment (1933–4) the plots were duplicated. The number of sets planted per plot varied from thirty-five to forty. At the time of planting all the tubers had sprouted, and although a few "misses" did subsequently occur in most of the plots there was no evidence that these were due to skin spot or that disinfection had reduced their number.

The crops were lifted at the end of September or the beginning of October a few weeks after the haulms had completely died down. The small-sized chats, blighted and unsound tubers were discarded. The remainder of the crop from each plot was clamped immediately where it was thought that conditions would be most suitable for the development of skin spot, the tubers being covered with a layer of straw before "earthing up". The clamps were not opened until the following spring when all the tubers were examined for skin spot. They were divided into two classes, (1) affected with skin spot, and (2) not affected, any tubers showing one or more skin-spot lesions being classified as affected. The results of these experiments (Table I) show that all the disinfectants employed, with the exception of formalin, produced a definite reduction

Table I. *Control of skin spot by tuber disinfection*, 1933–5 *experiments*

Treatment		1933–4 Sharpe's Express		1933–4 Kerr's Pink		1934–5 Sharpe's Express	
		Total crop lb.	% affected with skin spot	Total crop lb.	% affected with skin spot	Total crop lb.	% affected with skin spot
Nil	*a*	54	81·5	—	—	—	—
	b	48	79·2	87	77·0	43·5	79·3
	Mean	51	80·4	—	—	—	—
0·1 % mercuric chloride, 90 min. steep	*a*	58	56·9	110	22·7	42	50·0
	b	51	33·3	—	—	—	—
	Mean	54·5	45·1	—	—	—	—
0·25 % organo-mercurial A, 15 min. steep	*a*	54·5	53·2	—	—	—	—
	b	47·5	50·5	114	14·9	—	—
	Mean	51	51·9	—	—	—	—
0·75 % organo-mercurial A, instantaneous dip	*a*	63·5	52·8	—	—	—	—
	b	48	20·8	—	—	—	—
	Mean	55·8	36·8	—	—	—	—
1·0 % ,,		—	—	—	—	44	54·5
1·5 % ,,	*a*	58	43·1	—	—	—	—
	b	62	43·5	—	—	—	—
	Mean	60	43·3	—	—	—	—
1·5 % organo-mercurial B, instantaneous dip		—	—	—	—	51·5	56·3
Formalin (1 : 240), 90 min. steep		—	—	109	83·9	—	—

in the amount of skin spot which developed. It was also noted that the incidence or severity of the disease was generally much less on the affected tubers grown from disinfected seed than on those grown from non-disinfected seed. Consequently, the reduction in the amount of skin spot due to disinfection was greater than that indicated by the tabulated results.

III. The time of disinfection

Although the results from the preliminary experiments were promising, nevertheless a larger reduction in the incidence of the disease was desirable, and in order to study the effect of varying the time of treatment a fuller investigation was commenced in 1935. Here it was decided to compare the efficiency of disinfection at two distinct times, viz.:

(*a*) Immediately before planting, i.e. disinfection of the seed tubers in the spring, the resultant crop *not* being disinfected before storage.

(*b*) Immediately after digging, i.e. the planting of *non-disinfected* seed tubers and the disinfection of the resultant crop before storage.

Two varieties of potato, Kerr's Pink and Sharpe's Express, were used, all the seed being selected as severely affected with skin spot. For (*a*) the seed tubers were disinfected about 1 week prior to planting, while for

(*b*) the resultant crop was disinfected immediately after lifting. Three methods of disinfection were employed:

(1) A 90 min. steep in a 0·1 % mercuric chloride solution.

(2) An "instantaneous" dip in a 1·5 % solution of organo-mercurial B.

(3) An "instantaneous" dip in a 1·0 % solution of organo-mercurial C, a modified form of organo-mercurial A used in the preliminary experiments.

The planting, lifting and storage of the crop were similar to the technique adopted for the preliminary experiments with the exception that each plot consisted of one drill 10 yd. in length, and that the same number of sets was planted in each plot. After storage in clamps during the winter the tubers were washed and examined for skin spot in the following spring (1936). They were then divided into three classes:

(*a*) Clean—those showing complete freedom from skin-spot lesions.

(*b*) Slightly diseased—those showing one to five skin-spot lesions per tuber.

(*c*) Severely diseased—those showing six or more skin-spot lesions per tuber.

The number and weight of tubers in each class were recorded, but as the data obtained in each case showed a close degree of correlation, tuber numbers only are recorded in Table II. Here the total number of tubers falling into classes (*a*), clean, and (*b*), slight skin spot, are also grouped together in a category designated as "commercially clean". It is quite possible that a few or even one lesion per tuber may in some cases cause severe injury to the young sprouts, and this may be particularly true when the infection is in the region of the eyes. In most of the tubers examined which belonged to the slight skin-spot class, however, the infection was observed at the "heel" end. Thus, for commercial purposes it would appear to be quite legitimate to regard such slightly affected tubers as "clean".

From these results it will be seen that disinfection of seed tubers a few weeks before planting again gave a definite reduction in the incidence of the skin spot which developed in the tubers of the resultant crop during storage. A much more efficient control of the disease, however, was obtained where the seed tubers were *not* disinfected but the resultant crop disinfected immediately after digging.

The efficacy of disinfection immediately after digging in preventing the development of skin spot during storage was confirmed during 1936–7. Potatoes of the varieties Arran Banner and Kerr's Pink grown on a farm in Co. Down from seed affected with skin spot and not disin-

Table II. *Control of skin spot by tuber disinfection, 1935–6 experiment*

Variety	Time of disinfection	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Sharpe's Express	Seed tubers *not* disinfected, resultant crop disinfected immediately after digging	Water, instantaneous dip		113	65·5	30·1	4·4	34·5	95·6
		0·1 % $HgCl_2$, 90 min. steep		137	1·5	21·9	76·6	98·5	23·4
		1·5 % organo-mercurial B, instantaneous dip		120	6·7	26·7	66·6	93·3	33·4
		1·0 % organo-mercurial C, instantaneous dip		96	1·1	3·1	95·8	98·9	4·2
,,	Seed tubers disinfected before planting, resultant crop *not* disinfected after digging	Nil		154	41·6	45·5	12·9	58·4	87·1
		0·1 % $HgCl_2$, 90 min. steep		134	4·5	32·8	62·7	95·5	37·3
		1·5 % organo-mercurial B, instantaneous dip		151	22·5	31·8	45·7	87·5	54·3
		1·0 % organo-mercurial C instantaneous dip		165	4·9	30·3	64·8	95·1	35·2
Kerr's Pink	Seed tubers *not* disinfected, resultant crop disinfected immediately after digging	Water, instantaneous dip		169	90·5	7·7	1·8	9·5	98·2
		0·1 % $HgCl_2$, 90 min. steep		168	28·5	30·5	41·0	71·5	59·0
		1·5 % organo-mercurial B, instantaneous dip		150	36·0	31·3	32·7	64·0	67·3
		1·0 % organo-mercurial C, instantaneous dip		168	0·6	4·8	94·6	99·4	5·4
,,	Seed tubers disinfected before planting, resultant crop *not* disinfected after digging	Nil	*a*	161	78·9	14·9	6·2	21·1	93·8
			b	161	78·2	14·3	7·5	21·8	92·5
			Mean	161	78·6	14·6	6·8	21·4	93·2
		0·1 % $HgCl_2$, 90 min. steep	*a*	176	35·8	38·1	26·1	64·2	73·9
			b	128	38·3	48·4	13·3	61·7	86·7
			Mean	152	37·0	43·3	19·7	63·0	80·3
		1·5 % organo-mercurial B	*a*	165	38·1	36·4	25·5	61·9	74·5
			b	162	29·0	30·9	40·1	71·0	59·9
			Mean	163·5	33·6	33·6	32·8	66·4	67·2
		1·0 % organo-mercurial C	*a*	203	29·5	35·5	35·0	70·5	65·0
			b	250	26·8	42·4	30·8	73·2	69·2
			Mean	226·5	28·2	38·9	32·9	71·8	67·1

fected before planting were harvested during October 1936. Quantities of ware and seed size of each variety were forwarded immediately to the Agricultural Research Institute, Hillsborough, where they were disinfected on the day after digging. After disinfection the tubers were thoroughly dried and stored. Two methods of storage were employed: (1) in a clamp, (2) in sprouting boxes in a potato house. In the clamp the experiment was arranged in duplicate, while in the sprouting boxes it was carried out in triplicate. The quantities of tubers used and the treatments given were as follows:

I. Clamp storage
   2 lots each 0·5 cwt.—no treatment—Control.
   2 „ „ „ "Instantaneous" dip in 1·5 % organo-mercurial B.
   2 „ „ „ "Instantaneous" dip in 0·5 % organo-mercurial C.

II. Stored in sprouting boxes
   3 lots each 21 lb. (i.e. 3 boxes)—no treatment—Control.
   3 „ „ „ „ „ "Instantaneous" dip in 1·5 % organo-mercurial B.
   3 „ „ „ „ „ "Instantaneous" dip in 0·5 % organo-mercurial C.

The examination for skin spot was made during the following May and June, the tubers being divided into three classes as in 1936. The results recorded in Tables III and IV show that tuber disinfection immediately after digging gave excellent control of skin spot. They also show that in the case of the non-disinfected tubers the incidence of severe skin-spot attack was much higher under the conditions of clamp storage than in the sprouting boxes. Disinfection also appears to be somewhat less efficient for tubers stored in sprouting boxes, this being especially marked in the slight skin-spot class.

Many of the tubers stored in the clamp were affected with a bacterial soft rot, probably due to attack by *Bacillus carotovorus* either directly or indirectly, with *Fusarium* rot, pit rot or blight (*Phytophthora infestans*) as forerunners. A count of the number of rotten tubers in the various lots indicated that disinfection had given a promising control. All the rotten tubers were discarded and were not included in the skin-spot results. Very few rotten tubers were present in the sprouting boxes and all the tubers were well sprouted.

That immediately after digging is the most satisfactory time for

Table III. *Control of skin spot by tuber disinfection, Kerr's Pink, 1936–7 experiment*

Method of storage	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Clamp	Nil	*a*	195	52·3	17·9	29·8	47·7	70·2
		b	86	72·1	14·0	13·9	27·9	86·1
		Mean	140	62·2	16·0	21·8	37·8	78·2
	1·5 % organo-mercurial B, instantaneous dip	*a*	232	9·9	14·2	75·9	90·1	24·1
		b	226	12·4	19·5	68·1	87·6	31·9
		Mean	229	11·2	16·8	72·0	88·8	28·0
	0·5 % organo-mercurial C, instantaneous dip	*a*	183	2·2	7·1	90·7	97·8	9·3
		b	203	1·0	5·9	93·1	99·0	6·9
		Mean	193	1·6	6·5	91·9	98·4	8·1
Sprouting boxes	Nil	*a*	92	47·8	28·3	23·9	52·2	76·1
		b	95	45·3	33·7	21·0	54·7	79·0
		c	101	35·6	46·5	17·9	64·4	82·1
		Mean	96	42·9	36·2	20·9	57·1	79·1
	1·5 % organo-mercurial B, instantaneous dip	*a*	90	25·6	35·6	38·8	74·4	61·2
		b	91	12·1	31·9	56·0	87·9	44·0
		c	92	27·2	30·4	42·4	72·8	57·6
		Mean	91	21·6	32·6	45·8	78·4	54·2
	0·5 % organo-mercurial C, instantaneous dip	*a*	89	3·4	18·0	78·6	96·6	21·4
		b	94	6·4	26·6	67·0	93·6	33·0
		c	78	1·3	17·9	80·8	98·7	19·2
		Mean	87	3·7	20·8	75·5	96·3	24·5

disinfection was again demonstrated in an experiment carried out during 1937–8, the results of which are shown in Table V. In addition to comparing the efficacy of various fungicides and confirming results previously obtained, one of the objects of this experiment was to determine the efficacy of tuber disinfection at various intervals after digging for the control of skin spot during storage. Tubers of the variety Kerr's Pink severely affected with skin spot were planted in land which had grown potatoes during 1935 and 1936. The 1935 crop was severely affected with the disease. Unfortunately, the 1936 crop was not retained in storage for skin-spot examination and, consequently, there is no record of the degree of attack on the tubers for this year.

In 1937 randomized plots each of one drill 9 yd. in length were used for the work and the various treatments, details of which are given in Table V, were carried out in duplicate. All the plots were harvested on 1 November, the produce of those requiring immediate treatment being disinfected on the same day. The tubers were stored in sprouting boxes for 3 days after treatment in order to ensure thorough drying, after which they were removed to a clamp. In the clamp the produce of the individual plots was separated by a thick layer of straw. The lots which

Table IV. *Control of skin spot by tuber disinfection, Arran Banner, 1936–7 experiment*

Method of storage	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Clamp	Nil	*a*	120	79·2	15·0	5·8	20·8	94·2
		b	134	74·6	14·9	10·5	25·4	89·5
		Mean	127	76·9	15·0	8·1	23·1	91·9
	1·5 % organo-mercurial B, instantaneous dip	*a*	180	6·1	16·7	77·2	93·9	22·8
		b	152	7·2	11·2	81·6	92·8	18·4
		Mean	166	6·7	13·9	79·4	93·3	20·6
	0·5 % organo-mercurial C, instantaneous dip	*a*	167	0·0	9·0	91·0	100·0	9·0
		b	175	1·1	4·6	94·3	98·9	5·7
		Mean	171	0·6	6·8	92·6	99·4	7·4
Sprouting boxes	Nil	*a*	85	30·6	35·3	34·1	69·4	65·9
		b	72	26·4	40·3	33·3	73·6	66·7
		c	87	20·7	32·2	47·1	79·3	52·9
		Mean	81	25·9	35·9	38·2	74·1	61·8
	1·5 % organo-mercurial B, instantaneous dip	*a*	71	9·9	26·8	63·3	90·1	36·7
		b	76	7·9	22·4	69·7	92·1	30·3
		c	76	17·1	21·1	61·8	82·9	38·2
		Mean	74	11·6	23·4	65·0	88·4	35·0
	0·5 % organo-mercurial C, instantaneous dip	*a*	70	0·0	12·9	87·1	100·0	12·9
		b	70	1·4	18·6	80·0	98·6	20·0
		c	71	1·4	16·9	81·7	98·6	18·3
		Mean	70	0·9	16·1	83·0	99·1	17·0

required disinfection at various intervals after digging were stored in a separate clamp from which they were removed as required, disinfected, and placed in the other clamp.

Examination for skin spot was made at the time of disinfection, but the disease was not observed until 8 weeks after digging when a few tubers were seen to be affected. Sixteen weeks after digging many tubers had become severely affected with the disease.

The clamp was opened in May 1938, and all the tubers washed and examined. The results, which are given in Table V, show that under the conditions of this experiment, the disinfection of the tubers 2 or 4 weeks after digging was as satisfactory in securing the control of skin spot during storage as when it was carried out on the day of digging. When a longer interval was allowed to elapse the results were not so satisfactory, and this is particularly marked where 16 weeks intervened between digging and treatment. It will also be seen that there were distinct differences in the efficiency of the various fungicides employed and, in this respect, the results largely confirm those obtained in previous experiments.

Table V. *Control of skin spot by tuber disinfection, 1937–8 experiment*

Time of disinfection	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Seed tubers *disinfected* before planting. Resultant crop *not* disinfected after digging	Nil	*a*	171	44·4	31·5	24·1	55·6	75·9
		b	181	61·9	25·4	12·7	38·1	87·3
		Mean	176	53·1	28·5	18·4	46·9	81·6
	0·5 % organo-mercurial C, instantaneous dip	*a*	203	34·5	33·0	32·5	65·5	67·5
		b	179	17·3	26·8	55·9	82·7	44·1
		Mean	191	25·9	29·9	44·2	74·1	55·8
	1·5 % organo-mercurial B, instantaneous dip	*a*	181	29·3	26·5	44·2	70·7	55·8
		b	218	42·2	34·4	23·4	57·8	76·6
		Mean	199	35·8	30·4	33·8	64·2	66·2
	0·1 % mercuro-chloride, 90 min. steep	*a*	180	19·4	27·2	53·4	80·6	46·6
		b	205	19·0	31·2	49·8	81·0	50·2
		Mean	192	19·2	29·2	51·6	80·8	48·4
Seed tubers *not* disinfected. Resultant crop *disinfected* immediately after digging (i.e. on the same day)	0·5 % organo-mercurial C, instantaneous dip	*a*	176	0·0	2·8	97·2	100·0	2·8
		b	184	0·5	9·8	89·7	99·5	10·3
		Mean	180	0·3	6·3	93·4	99·7	6·6
	1·5 % organo-mercurial B, instantaneous dip	*a*	166	5·4	16·0	78·6	94·6	21·4
		b	181	7·2	19·3	73·5	92·8	26·5
		Mean	173	6·3	17·7	76·0	93·7	24·0
	0·1 % mercuro-chloride, 90 min. steep	*a*	183	3·3	14·2	82·5	96·7	17·5
		b	207	0·5	17·9	81·6	99·5	18·4
		Mean	195	1·9	16·1	82·0	98·1	18·0
	1·5 % formalin, instantaneous dip	*a*	153	27·5	36·6	35·9	72·5	64·1
		b	165	18·8	55·2	26·0	81·2	74·0
		Mean	159	23·2	45·9	30·9	76·8	69·1
	Proprietary dust applied at rate of 4 lb. per ton potatoes	*a*	170	45·3	26·5	28·2	54·7	71·8
		b	175	54·9	19·4	25·7	45·1	74·3
		Mean	172	50·1	23·0	26·9	49·9	73·1
Seed tubers *not* disinfected. Resultant crop disinfected at varying intervals after digging	2 weeks after digging, 0·5 % organo-mercurial C, instantaneous dip	*a*	170	1·2	7·6	91·2	98·8	8·8
		b	193	0·5	4·7	94·8	99·5	5·2
		Mean	181	0·8	6·2	93·0	99·2	7·0
	4 weeks after digging, 0·5 % organo-mercurial C, instantaneous dip	*a*	201	2·5	5·0	92·5	97·5	7·5
		b	171	1·2	7·0	91·8	98·8	8·2
		Mean	186	1·9	6·0	92·1	98·1	7·9
	8 weeks after digging, 0·5 % organo-mercurial C	*a*	194	10·3	16·5	73·2	89·7	26·8
		b	211	4·7	11·8	83·5	95·3	16·5
		Mean	202	7·5	14·2	78·3	92·5	21·7
	16 weeks after digging, 0·5 % organo-mercurial C	*a*	186	41·9	28·5	29·6	58·1	70·4
		b	187	20·3	28·9	50·8	79·7	49·2
		Mean	186	31·1	28·7	40·2	68·9	59·8

IV. The time of digging and incidence of skin spot

During 1937–8 an experiment was carried out to determine whether the time of digging the crop would have any effect on the amount of skin spot which subsequently developed in storage. Seed tubers of the variety Kerr's Pink were selected as severely affected with skin spot, and on 17 May 1937 were used for the planting of twenty plots, each plot consisting of one drill of 9 yd. Twenty sets were planted in each plot. Five diggings were made at different dates during the season, the first

Block C	1st digging	5 August	3rd digging	1 October	Block A
	3rd ,,	1 October	5th ,,	29 November	
	4th ,,	1 November	2nd ,,	26 August	
	2nd ,,	26 August	4th ,,	1 November	
	5th ,,	29 November	1st ,,	5 August	
Block D	3rd ,,	1 October	5th ,,	29 November	Block B
	5th ,,	29 November	2nd ,,	26 August	
	1st ,,	5 August	4th ,,	1 November	
	4th ,,	1 November	3rd ,,	1 October	
	2nd ,,	26 August	1st ,,	5 August	

Text-fig. 1.

on 5 August when the tubers were small and immature, and while the foliage was still growing vigorously. The fifth and last digging was made on 29 November, the haulms having completely died down about 7 weeks previously. Skin spot was not observed on the tubers during any of the diggings. In October 1936 the disease was, however, noted by the writers at the time of lifting tubers of the same variety.

The lay-out of the plots and the dates of digging are given in Text-fig. 1. For each digging four of the plots were lifted, one plot being selected at random from each of the four blocks A, B, C and D. The small-sized chats, blighted and unsound tubers were discarded. The produce of the two plots from blocks A and C were thoroughly mixed and then divided into two lots of equal weight. One lot was disinfected immediately, an "instantaneous" dip in 0·5 % organo-mercurial C being employed, while the second lot was not disinfected. The produce of the two plots from blocks B and D received similar treatment. After disin-

fection all the tubers, disinfected and non-disinfected, were treated in a like manner. They were placed one layer deep in sprouting boxes and stored in a well-ventilated house for 3–4 days. After this period they were stored in a clamp, each lot being covered with a thick layer of straw before "earthing up". The tubers were removed from the clamp

Table VI. *The time of digging and incidence of skin spot, 1937–8 experiment*

Date of digging and disinfection with 0·5 % organo-mercurial C			Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
5 Aug. 1937	Untreated	*a*	146	48·6	28·1	23·3	51·4	76·7
		b	158	43·7	29·7	26·6	56·3	73·4
		Mean	152	46·2	28·9	24·9	53·8	75·1
	Treated	*a*	158	0·0	4·4	95·6	100·0	4·4
		b	195	0·0	4·1	95·9	100·0	4·1
		Mean	176	0·0	4·3	95·7	100·0	4·3
26 Aug. 1937	Untreated	*a*	230	63·9	24·8	11·3	36·1	88·7
		b	199	53·8	27·1	19·1	46·2	80·9
		Mean	214	58·8	26·0	15·2	41·2	84·8
	Treated	*a*	220	0·0	2·3	97·7	100·0	2·3
		b	224	0·0	8·5	91·5	100·0	8·5
		Mean	222	0·0	5·4	94·6	100·0	5·4
1 Oct. 1937	Untreated	*a*	162	65·4	23·5	11·1	34·6	88·9
		b	197	54·8	30·5	14·7	45·2	85·3
		Mean	179	60·1	27·0	12·9	39·9	87·1
	Treated	*a*	172	0·0	9·3	90·7	100·0	9·3
		b	209	1·0	3·8	95·2	99·0	4·8
		Mean	190	0·5	6·6	92·9	99·5	7·1
1 Nov. 1937	Untreated	*a*	238	58·4	18·9	22·7	41·6	77·3
		b	217	62·7	21·7	15·6	37·3	84·4
		Mean	227	60·6	20·3	19·1	39·4	80·9
	Treated	*a*	189	0·0	9·0	91·0	100·0	9·0
		b	216	0·0	4·6	95·4	100·0	4·6
		Mean	202	0·0	6·8	93·2	100·0	6·8
29 Nov. 1937	Untreated	*a*	183	45·9	31·1	23·0	54·1	77·0
		b	175	45·7	26·3	28·0	54·3	72·0
		Mean	179	45·8	28·7	25·5	54·2	74·5
	Treated	*a*	183	1·6	7·7	90·7	98·4	9·3
		b	194	1·5	9·8	88·7	98·5	11·3
		Mean	188	1·5	8·8	89·7	98·5	10·3

the following May and the method of examination and classification for skin spot adopted was similar to that employed in 1936 and 1937. The results, which are given in Table VI, show that severe skin spot developed in all the non-disinfected lots of tubers, and that there was little or no difference in the incidence of the disease irrespective of the time of digging. For all the diggings, disinfection immediately after lifting gave excellent control of skin spot.

V. Discussion

General. O. pustulans is a soil-borne fungus and is generally assumed to be present in all soils which are suitable for its maintenance. Scottish records (Scottish Journal, 1932) show that skin spot occurred in tubers produced by non-affected seed planted in land which had been in grass for at least 20 years before cropping, and which had received no farmyard manure. This and similar evidence would suggest that in such cases the amount of infection present on the seed at planting or the disinfection of the seed prior to planting would have little, if any, effect upon the disease in the resultant crop. Nevertheless, the results of this investigation indicate that, at least in some soils and under the experimental conditions obtaining, the disinfection of affected tubers before planting does give a certain measure of control. At the same time a larger reduction in the incidence of the disease than that obtained by disinfection immediately before planting is highly desirable. The prevention of the development of the disease during storage irrespective of the conditions or the amount of infection present in the soil in which the tubers are grown is the ideal objective, and this was more nearly attained by disinfection of the tubers immediately after digging. Although the experiments dealing with disinfection immediately after digging are limited in their range in so far as soil type, potato variety and climatic conditions are concerned, yet the results obtained have been very encouraging and suggest that this method is worthy of a wider investigation.

Method of disease assessment. The incidence of the disease in all tuber samples was assessed on the presence or absence of skin-spot lesions on the tubers. The subsequent behaviour of the tubers with regard to sprouting and plant establishment in the field was not closely studied. This was chiefly due to the fact that seed tubers of the varieties used—Sharpe's Express, Kerr's Pink and Arran Banner—may be severely affected with skin spot without subsequent damage to sprouting or establishment. Various samples of tubers of all three varieties severely affected with the disease and retained for planting gave rise to healthy vigorous sprouts and excellent plant establishment. It would, probably, have been more satisfactory to have used such varieties as King Edward and Majestic in which non-sprouting and poor establishment due to skin spot have been frequently recorded, and to have assessed the incidence of the disease under three heads, i.e. tuber lesions, sprouting, and establishment; but it was not possible to obtain suitable supplies of these varieties heavily infected with the disease. On the whole, however, it

would appear justifiable to assume that the method of assessment adopted gives a true index of the incidence of the disease, and that methods of control giving satisfactory results here would be almost if not equally successful with the more susceptible varieties.

Relative values of fungicides. The most satisfactory method of treatment was found to consist of the "instantaneous" dipping (0·5–1·0 min.) of the tubers in a solution of an organo-mercury compound. The three fungicides of this type (A, B and C) used in the present work were of a proprietary nature, and particularly good results were obtained by the use of organo-mercurial C in aqueous solution at a strength of 0·5 %. Promising results were also obtained by steeping the tubers for 90 min. in a 0·1 % solution of mercuric chloride, but the tediousness of this method proved to be a disadvantage. The proprietary dust fungicide used in 1937 proved to be quite ineffective under the experimental conditions obtaining. The use of formalin was not encouraging, and in most cases little or no control of the disease was secured by this method.

The action of fungicides relative to the time of infection and the development of the disease. If it be assumed that formalin is lethal for all latent infection present on the tuber or in the soil adhering to the tuber at the time of digging, then the reason for its ineffectiveness may be found to lie in the time of infection of the tuber and the subsequent rate of development of the disease, problems which have not yet been fully elucidated. It is possible that infection occurs before the normal time of digging, a conception which is rather favoured by results obtained from the time of digging experiment 1937–8, and that the fungus remains latent in the lenticels and periderm region for some time before undergoing further development. Considerable evidence is available which indicates that in the case of diseases of ripening and stored fruit there may be a pronounced lapse of time between infection and the development of disease (Baker, 1938). If such be the case the killing of an infective principle present on or near the surface of the tuber at digging time would not ensure disease control. On the other hand, preparations of mercury used as fungicides did secure definite control, and this may be explained by their action within the lenticels and periderm region thereby killing the parasite resting there before its further development. Experiments carried out in 1937–8 showed clearly that even disinfection with organo-mercurial C gave only partial control when carried out at a date later than about 8 weeks after the digging of the crop, thus suggesting that this control measure is non-effective when the parasite has developed beyond a certain stage. Evidence that fungicidal action within the peri-

derm region is possible when organo-mercury compounds are used is provided by the fact, that when slightly immature tubers are dipped or if the fungicide is too strong, then the skin of the tuber may be killed and slough off leaving the exposed tissue beneath distinctly browned. In the case of very young tubers this may result in loss of water by the tuber and its becoming soft and india-rubber like. A further advantage which may accrue from the use of mercury as a fungicide is the fact that the material dries and persists as a coating on the surface of the tuber thereby preventing infection from extraneous sources during storage, whereas the more temporary effect of formalin does not provide such protective action.

Digging time and the incidence of skin spot. The earlier digging of potato crops intended for use as "seed" is a practice which is probably deserving of more attention than it has received in the past. It appears to be making headway in Holland where it is recommended in particular as an aid towards keeping potato stocks freer from virus infection. The better weather conditions which normally obtain in the early autumn with the consequent lifting of cleaner and more presentable tubers is also a point worthy of consideration by the "seed" grower. In 1937–8 an attempt was made to determine the effect of the time of digging upon the incidence of skin spot in the resultant crop. The results obtained show that the disease developed in all samples irrespective of the earliness of digging, although tuber disinfection on the same day as digging gave excellent control in every case. It must, however, be pointed out that these results hold for only one variety in one year under one set of soil conditions and, as has already been stressed, when the variability of these three factors is taken into account, too much emphasis cannot be laid upon these findings as general principles. Although no skin-spot lesions were observed on any of the tubers at any of the digging times during the experiment, yet in a previous season a very slight attack of the disease was observed at the time of lifting a crop of the same variety.

Optimum time for disinfection. All things considered, the optimum time for tuber disinfection for skin-spot control is as soon as possible after digging. Although the results of 1937–8 indicate that treatment need not necessarily be carried out immediately, they also show that when carried out at an interval of more than 8 weeks after digging its effectiveness is substantially decreased. The selection and disinfection of "seed" at digging time serves a dual purpose in that it secures the prevention of the development of skin spot during storage and ensures the planting of comparatively disease-free seed. Further, digging time is also the

optimum time for disinfection when tuber diseases such as common scab (Cairns *et al.* 1936) and blight (Greeves, 1937) are taken into consideration. Some evidence has also been obtained and is here recorded of disinfected tubers proving to be less susceptible to storage diseases. Lastly, as the same technique is applicable in all cases, it would appear to be possible by the same treatment, i.e. disinfection of the seed tubers at the time of digging, to control not only skin spot but a group of diseases of the seed tuber, many of which are transmissible to the ensuing crop.

VI. Summary

1. Experiments carried out during 1933–8 with the object of investigating the control of skin spot (*Oospora pustulans* Owen & Wakef.) by seed-tuber disinfection are described.

2. Compounds of mercury were effective when used in solution as steeps. Formalin and a proprietary dusting compound proved to be non-effective. Proprietary organo-mercury compounds gave the most promising results, and were satisfactory when used as "instantaneous" dips (0·5–1·0 min.); mercuric chloride solution was efficient but the 90 min. dipping period employed made the method too tedious.

3. The time at which the treatment is carried out is of great importance in determining the measure of control obtained. Disinfection of the seed at the time of digging was far more effective than immediately before planting. If too long an interval (more than 8 weeks) is allowed to elapse between digging and disinfection, the value of the treatment is substantially reduced.

4. A study of the time of digging the crop upon the incidence of the disease, made during one year only, indicated that for that year early digging did not reduce the amount of the disease which developed during subsequent storage. Disinfection carried out at each of the five diggings gave satisfactory control in each case.

5. The method of treatment recommended is the same as that previously suggested for the control of common scab (*Actinomyces scabies* (Thaxt.) Güss.) and blight (*Phytophthora infestans* (Mont.) de Bary). Some evidence was obtained of its also being effective against storage diseases. It would, therefore, appear that disinfection of seed tubers at the time of digging may prove effective against a group of tuber diseases many of which are transmissible to the resultant crop.

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REFERENCES

Baker, R. E. D. (1938). Studies in the pathogenicity of tropical fungi. II. The occurrence of latent infections in developing fruit. *Ann. Bot., Lond.*, **2**, N.S. 919–31.

Cairns, H., Greeves, T. N. & Muskett, A. E. (1936). The control of common scab (*Actinomyces scabies* (Thaxt.) Güss.) of the potato by tuber disinfection. *Ann. appl. Biol.* **23**, 718–42.

Greeves, T. N. (1937). The control of blight (*Phytophthora infestans*) in seed potatoes by tuber disinfection. *Ann. appl. Biol.* **24**, 26–32.

Ministry of Agriculture and Fisheries (1937). Skin spot and silver scurf of potatoes. *Adv. Leafl. Minist. Agric. Fish., Lond.*, no. 279.

Scottish Journal (1932). Skin spot and blindness in seed potatoes. *Scot. J. Agric.* **15**, 191–6.

EXPLANATION OF PLATE XXIV

The photographs show representative portions of the crops of the variety Sharpe's Express (1935–6 experiments). In each case the crop sample is graded into three classes, viz.: A, severely affected; B, slightly affected; and C, clean tubers. The photographs were taken in June 1936.

Fig. 1. Crop grown from *non*-disinfected seed; resultant crop *not* disinfected after digging.

Fig. 2. Crop grown from seed disinfected before planting (1·0 % organo-mercurial C); resultant crop *not* disinfected after digging.

Fig. 3. Crop grown from *non*-disinfected seed; resultant crop disinfected immediately after digging (1·0 % organo-mercurial C).

Fig. 4. Crop grown from *non*-disinfected seed; resultant crop disinfected immediately after digging (1·5 % organo-mercurial B).

(*Received* 11 *January* 1939)

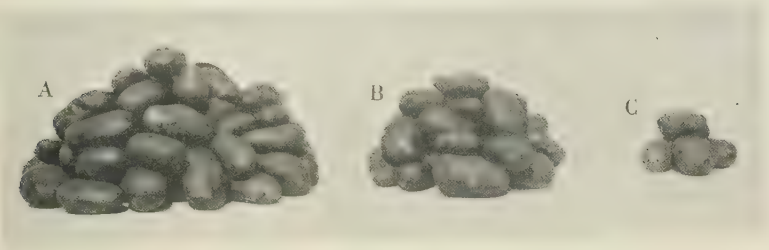

Fig. 1.

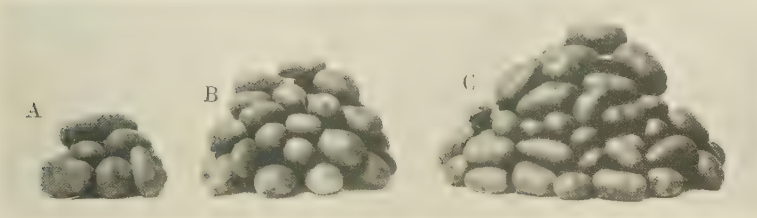

Fig. 2.

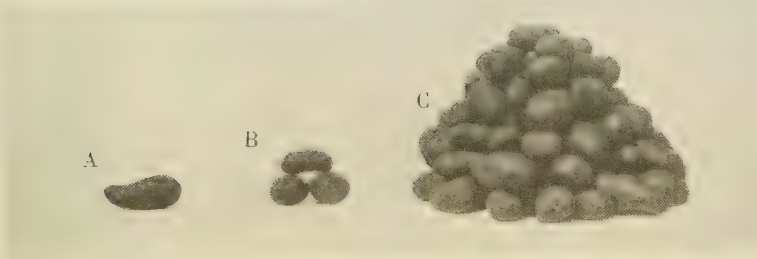

Fig. 3.

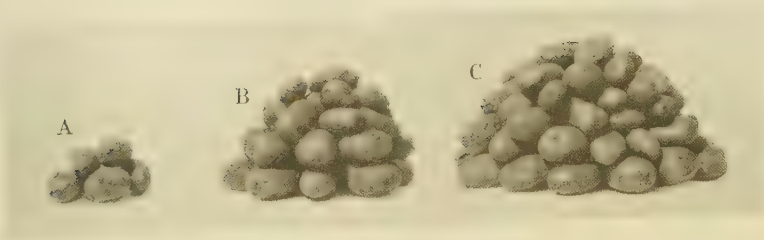

Fig. 4.

GREEVES AND MUSKETT.—SKIN SPOT (*OOSPORA PUSTULANS* OWEN & WAKEF.) OF THE POTATO, AND ITS CONTROL BY TUBER DISINFECTION (pp. 481–496)

SUCCESSION OF FUNGI DECOMPOSING WHEAT STRAW IN DIFFERENT SOILS, WITH SPECIAL REFERENCE TO *FUSARIUM CULMORUM*

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(With 2 Text-figures)

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I. Introduction

It has long been known that a succession of fungi develops in the underground parts of a plant after infection by a primary parasite. This succession was studied by Hildebrand (1934) for strawberry root rot, by Koch (1935) for tobacco root rot, and further investigated by Hildebrand & Koch (1936) by means of direct microscopical examination of infected roots. The precise extent to which secondary parasites increase the damage done by the primary parasite is uncertain, but it was found by Hansen (1929) that pink-root infection of onions by *Phoma terrestris* was aggravated by simultaneous inoculation with *Fusarium malli*, a secondary parasite rather regularly following *Phoma terrestris* in natural pink-root infection. Again, Geach (1933) found that whereas *Urocystis tritici* alone caused 3·6 % seedling blight in wheat seedlings, and *Fusarium culmorum* 1·8 % under the particular field conditions of his experiments and with his particular isolations of these fungi, yet where the two fungi were used together a mortality of 37·8 % of seedlings was the result. Attention was first drawn by Fawcett (1931) to the fact that mixed infections of fruit and other above-ground parts of plants are more common than was formerly supposed.

Experience in this laboratory has shown repeatedly that it is difficult to isolate *Ophiobolus graminis* from the stem bases of wheat plants thoroughly infected with this fungus, and heavily encrusted with typical plate mycelium. Attention was attracted to the frequent occurrence of *Fusarium culmorum* colonies, which occasionally constituted as much as 90 % of those developing from the surface-sterilized stem bases plated out on acidified potato-dextrose agar. The results obtained from plating out *Ophiobolus*-infected stem bases of wheat and barley plants collected from seven different crops in the 1937 season are given in Table I.

Table I. *Fungi isolated from surface-sterilized stem bases of wheat and barley showing typical plate mycelium of* Ophiobolus graminis

	No. of straws plated out	% straws yielding colonies of *O. graminis*	% straws yielding colonies of *F. culmorum*
Wheat, from Reading, Berks	20	35	15
,, ,, Harnhill, Wilts	92	4	38
,, ,, Castle Ashby, Northants	36	3	6
,, ,, Wrington, Somerset	12	0	67
Barley, from Fulbourn, Cambs	40	5	23
,, ,, Tunstead, Norfolk	16	0	63
,, ,, Bridgham, Norfolk	44	0	34

Taking into consideration the well-established reputation of *Ophiobolus graminis* as a vigorous primary parasite, and also the fact that the diseased plants showed external mycelium of this fungus alone, it is difficult to resist the conclusion that *Fusarium culmorum* was here present in the capacity of a secondary parasite. At the same time there is no intention of calling in question either the capacity of *F. culmorum* for causing a severe seedling blight of cereals (Simmonds, 1928; Bennett, 1928; Blair, 1937), or the fact that it is often the only fungus associated with the whiteheads condition in England (Bennett, 1928; Russell, 1932), Canada (Simmonds, 1928; Greaney & Machacek, 1935), the U.S.A. (Tu, 1929), Australia (Geach, 1933) and elsewhere.

Further light was thrown upon the association of *F. culmorum* with the wheat plant by the observations of Samuel & Greaney (1937), who isolated the fungus from surface-sterilized root and crown pieces taken from the bases of normal wheat plants showing no disease symptoms. A survey was made from three fields in different localities, and samples taken at intervals of about a fortnight from heading until after harvest. The percentage of *F. culmorum* isolations obtained from two out of the three fields increased in the later samplings, and was greatest in that made from the stubble after harvest. Tests on the wheat seedlings in the

glasshouse showed that the pathogenicity of these isolates of *F. culmorum* was represented by an average disease rating (Greaney & Machacek, 1935) of 21 % for thirteen isolates of the fungus. Samuel & Greaney call attention to the earlier work of Broadfoot (1934), who obtained 20–60 % of *F. culmorum* in many thousands of isolations from wheat plants taken at random from a series of rotation plots. In conclusion, they comment upon their work as follows: "It is evident that the fungus must have been present in the soil from which these healthy wheat plants were taken, exerting no appreciable parasitic effect and entering the root systems, along with other fungi only as the roots began to lose vitality after the flowering of the crop. There was then a still further development of the fungus on the stubble, after the crop had been cut. This is in contrast to fairly numerous cases reported, especially from the north of England, of appreciable damage to wheat by the fungus *F. culmorum*."

These observations, taken in conjunction, suggested that if *F. culmorum* behaved as a regular secondary parasite of plants infected by *Ophiobolus graminis*, and as a weak primary parasite of normally senescent plants, it might also be able to colonize the dead roots and stem bases of wheat stubble left in the ground after harvest. It seemed possible that this fungus might also play an important part in the normal decomposition of threshed wheat straw when buried in the soil. An investigation into the succession of soil fungi colonizing such buried wheat straw has therefore been made, and forms the subject of the present communication.

II. Methods

Wheat straw for use in these experiments was selected from the upper parts of healthy plants, and cut into pieces about 1 in. in length. One set of straws was used unsterilized so as to form an entirely natural substratum. A second set of straws was sterilized by autoclaving for 30 min. at 1 atm. pressure in a 2 % solution of sodium nitrate. This concentration of sodium nitrate was calculated to give 1 g. of nitrogen per 100 g. of dry straw, it having been found that 1 g. of dry straw takes up 3 c.c. of water. Thus, two different substrata were employed, one of them the natural unsterilized straw and the other sterilized straw with sufficient nitrogen added to ensure complete decomposition when buried in the soil.

The soils employed in the first experiment were as follows:

Rothamsted. Flinty clay loam, *p*H 6·8; collected from the field, February 1938.

Allotment. Similar soil to the above, but better supplied with organic matter as a result of allotment cultivation for more than 70 years, *p*H 6·8; collected from the field, February 1938.

Downham. Black fen soil from Norfolk, *p*H 7·6; collected from the field in July 1937, and stored in bag until February 1938.

Compost. Glasshouse soil prepared by composting together 2 parts of grass sod and loam and 1 part of stable manure, stacked for 18 months, *p*H 6·8; this sample was taken from permanent glasshouse stock.

Woburn. Light sandy loam from the Woburn Experimental Station, *p*H 6·1; collected July 1937, and stored in bag until February 1938.

For the second experiment, fresh collections of the Rothamsted, Allotment and Woburn soils were made from the field, and the glasshouse compost was again taken from the permanent stock. In this experiment the Downham soil was replaced by one from Whitchurch:

Whitchurch. Clay loam overlying chalk subsoil, *p*H 8·1; collected July 1937, and stored in bag until May 1938.

The experiments were set up as follows: The soils were not air-dried, but were passed through a $\frac{1}{4}$ in. sieve to remove large lumps. Four-inch pots were used as containers: twenty-five straws were buried in each pot in five layers of five each in the different soils contained in the pots. After filling, the pots were well watered and incubated in a lightly covered wooden box over a layer of moist sand. The box was housed in the laboratory and the pots of soil kept moist by occasional waterings. The laboratory temperature fluctuated between 17 and 22° C. during the period of the experiments.

A sample of two pots was taken from each soil series at weekly intervals in Exp. I and at fortnightly intervals in Exp. II; one pot of each sample contained untreated and the other the treated straw. The straws of each sample were washed free of soil over a sieve, and further cleaned by shaking in several changes of water in a 500 c.c. conical flask. The straws were surface-sterilized by shaking for a period of 45–60 sec. in 1/1000 aqueous mercuric chloride solution, followed by a washing in six changes of sterile tap water. The surface sterilized straws were plated out (five per dish) on acidified potato-dextrose agar, adjusted to *p*H 5·0, and the plates incubated at 25° C. for a fortnight before examination of the colonies.

III. Experimental

(*a*) *Colonization of the straws by soil fungi*

Detailed records are given, in graphical form, only of *Fusarium culmorum*, *Mucor* spp. and *Penicillium* spp. These types, in virtue of their abundance, appeared to be dominant organisms in the earlier or later stages of straw colonization. The occurrence of other organisms was either too infrequent or too erratic to be worth recording in detail, but the genera included *Cylindrocarpon*, *Stemphylium*, *Gliocladium*, *Trichoderma*, *Pythium*, *Dicarpella*, *Alternaria*, *Geomyces*, *Aspergillus*, *Pullularia* and *Sordaria*. Two or even three fungal colonies not infrequently developed from a single piece of straw. Indeed, it must be emphasized that the fungi isolated from the straws in these experiments are not necessarily those dominant in the actual decomposition of the straw, but may merely be those most easily able to develop into colonies under the particular

conditions employed. It is possible that, with different methods of surface sterilization and incubation of the straws, somewhat different results might have been obtained.

In order to simplify the presentation of the numerous data, it was decided to graph the behaviour of *Fusarium culmorum*, *Mucor* spp., and *Penicillium* spp., respectively, as mean percentage occurrence in the five soils of each experiment. Thus each curve in Figs. 1 and 2 represents the results from five soils, of which an average has been made for graphical presentation. The five soils of Exp. I (Fig. 1) were Rothamsted, Allotment, Woburn, Compost and Downham; fresh collections of the first four soils were made for Exp. II (Fig. 2), but Downham was replaced by Whitchurch soil. Since these experiments are concerned especially with the occurrence of *F. culmorum*, additional data concerning this fungus are given in Table II, which shows the maximum percentage of straws yielding colonies of *F. culmorum* in the five soils of each experiment.

Table II. *Maximum percentage straws yielding colonies of* F. culmorum

	Soils					
	Roth-amsted	Allot-ment	Woburn	Compost	Downham	Whit-church
Exp. I:						
Untreated straws	95	90	85	90	75	—
N-treated straws	100	100	80	75	80	—
Exp. II:						
Untreated straws	36	56	48	36	—	56
N-treated straws	40	78	40	5	—	78

The behaviour of *F. culmorum*, *Mucor* spp., and *Penicillium* spp. (Figs. 1, 2) may be considered in sequence.

Fusarium culmorum. Both in Exps. I and II, the percentage of straws yielding *F. culmorum* reaches a maximum in the early stages of colonization of the straws and then declines. The nitrogenous treatment of the straws does not appear appreciably to affect the height of the maximum reached by *F. culmorum*, but hastens both the attainment of the maximum, and, particularly, the decline from the maximum. Autoclaving and nitrogen treatment thus appear to hasten the replacement of *F. culmorum* by other organisms, notably *Penicillium* spp. In Exp. II, the maximum attained by *Fusarium culmorum* is lower than that reached in Exp. I. From these two experiments, therefore, it would appear that *F. culmorum* declines in amount and activity with continued incubation of the soil, either in the field or in the store.

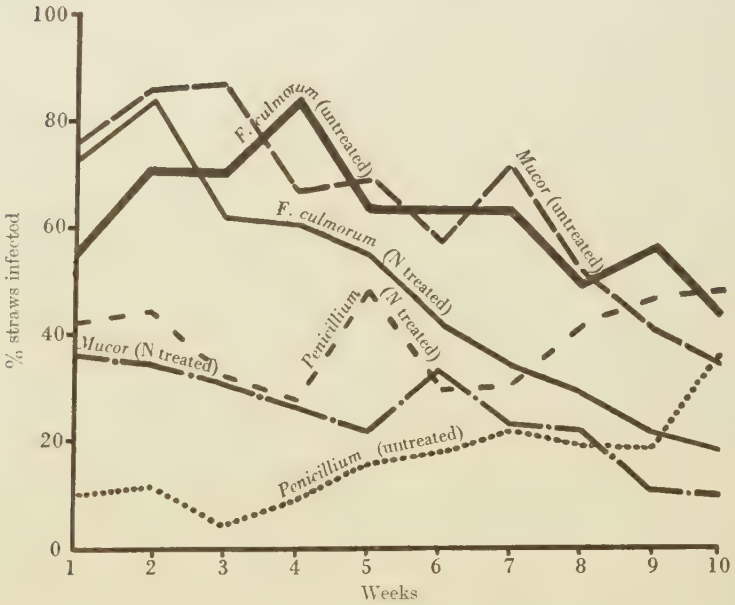

Fig. 1. Sequence of fungal development on untreated and nitrogen-treated straws buried in the soils of Exp. I.

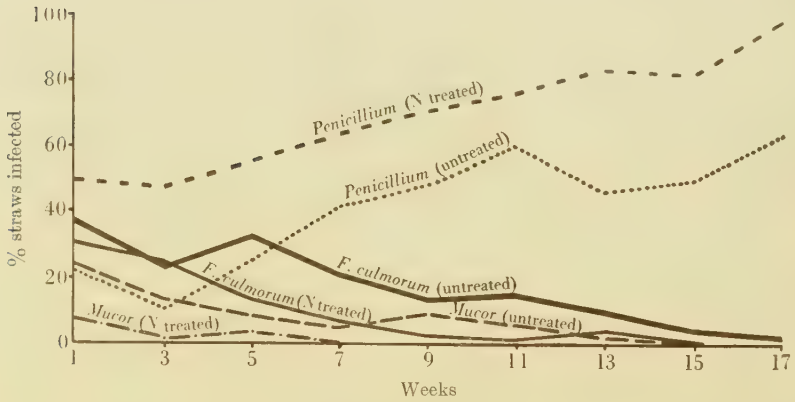

Fig. 2. Sequence of fungal development on untreated and nitrogen-treated straws buried in the soils of Exp. II.

Mucor spp. The general behaviour of *Mucor* is similar to that of *Fusarium culmorum*, inasmuch as its percentage occurrence declines from a maximum reached early in Exp. I; this initial maximum is a much lower one in Exp. II, suggesting that the activity of this fungus has also declined with incubation of the soil in field or store. The activity of *Mucor* is decidedly depressed by the nitrogen treatment of the straws in both experiments.

Penicillium spp. The behaviour of *Penicillium* is opposite to that of *Fusarium culmorum* and *Mucor*. Percentage occurrence rises from the early to the latter part of Exp. I; initial numbers are much higher in Exp. II, suggesting that the fungus has increased in relative importance with continued incubation of the soil in field or store. *Penicillium*, moreover, is decidedly encouraged by the nitrogen treatment of the straws. In all three respects just considered, therefore, the behaviour of *Penicillium* is the reverse of that displayed by *Fusarium culmorum* and *Mucor*.

General conclusions. These results emphasize the importance of *Fusarium culmorum* and *Mucor* spp. in the early stages of straw colonization by fungi, and of *Penicillium* spp. in the latter stages. The autoclaving in 2 % solution of sodium nitrate has exerted a pronounced effect upon the sequence of fungal colonization, apparently by encouraging the development of *Penicillium* spp. at the expense of *Fusarium culmorum* and *Mucor* spp. The replacement of *Fusarium culmorum* and *Mucor* spp. by *Penicillium* spp. that has taken place inside the buried straws also seems to have taken place in the soils with the advancement of season from winter (February) to early summer (May), as can be seen by comparing the results of Exps. I and II respectively.

(*b*) *Identification and pathogenicity tests of* Fusarium culmorum *isolates*

Identification. The different isolates from Exps. I and II were compared with a type culture of *F. culmorum* originally supplied by Dr F. T. Bennett to the Imperial College of Science and Technology, and with Bennett's (1928) published description of *F. culmorum* isolates from British cereals. Included in this comparison was an isolate made in this laboratory from diseased wheat roots. The different isolates were grown on potato-dextrose agar and the cultures were 30 days old when the spore measurements were made. Ten conidia in each of the groups, 3-, 4-, and 5-septate, were measured for each isolate, and the mean lengths and breadths are given in Table III. An analysis of variance (Fisher, 1936)

made on the complete data shows that differences in spore length between the different isolates are fully significant, but that on these data, at least, it is not possible to demonstrate any connexion between spore length and pathogenicity, since the two isolates obtained in the first instance as pathogens fall well within the range covered by the saprophytic isolates.

Table III. *Mean length of ten spores of each isolate in μ*

No. of isolate...	1	2	3	4	5	6	7	8	9	10	Type culture (Bennett)	Isolate from wheat roots
3-septate	26·3	29·4	31·7	27·4	30·3	30·7	30·1	30·4	31·6	28·3	26·5	30·7
4-septate	30·8	32·4	35·6	31·9	34·3	35·2	33·1	37·2	34·3	32·8	32·7	34·2
5-septate	34·4	35·4	39·7	33·7	39·0	37·3	34·9	41·5	37·6	36·4	34·5	37·3

Mean breadth of 10 spores of each isolate in μ

No. of isolate...	1	2	3	4	5	6	7	8	9	10	Type culture (Bennett)	Isolate from wheat roots
3-septate	7·4	7·0	7·3	7·4	7·4	6·9	7·7	7·5	7·8	7·7	7·2	7·4
4-septate	7·9	7·2	7·5	7·6	7·2	7·1	7·4	7·3	7·8	7·8	7·5	7·2
5-septate	7·9	7·5	6·9	7·2	7·1	7·3	7·5	7·2	8·2	7·7	7·5	7·2

Pathogenicity. Pathogenicity tests to establish the identity of the fungus were done with Little Joss wheat seedlings grown in 4 in. pots of sterilized sand. The agar-disk method of inoculation employed by Garrett (1936) for *Ophiobolus graminis* was used, and selected single-spore isolates from Exps. I and II were grown on potato-dextrose agar (*p*H 5·0) for the provision of suitable inoculum. Five presoaked wheat seeds were planted per pot, and the pots kept outside in the open. The first test, of isolates from Exp. I, was made during the period 4–25 May (mean air temperature 10° C.), and the second test, of isolates from Exp. II, during the period 30 July to 19 August (mean air temperature 16° C.). The seedlings were washed out and the number of infected seedlings and individual roots determined by inspection in water over a white background.

The twenty isolates employed in the first test were taken in pairs from the ten consecutive samplings of Exp. I. Five isolates were taken from each of the first three samplings of Exp. II for the second test, in which was included for comparison five isolates from infected wheat roots, and the type culture of *Fusarium culmorum* (Bennett's). The results of these two pathogenicity tests are set out in Tables IV and V.

Table IV. *Pathogenicity test of* F. culmorum *isolates from Exp. I*

Isolates of *F. culmorum*	No. of seedlings used in test	No. killed before emergence	No. killed after emergence	% infected roots in remaining seedlings	Total % roots infected in all seedlings
1	10	4	2	60	84
2	10	2	3	66	83
3	10	5	1	66	86
4	10	5	1	70	88
5	10	3	1	60	76
6	10	3	1	77	86
7	10	5	1	82	93
8	10	2	1	56	69
9	10	5	2	70	91
10	10	7	2	100	100
11	10	3	0	63	74
12	10	3	0	45	62
13	10	5	1	82	93
14	10	6	1	85	96
15	10	6	2	85	99
16	10	4	1	52	76
17	10	5	0	43	72
18	10	5	3	95	99
19	10	6	0	36	74
20	10	7	1	36	87

Table V. *Pathogenicity test of* F. culmorum *isolates from Exp. II*

	Isolates of *F. culmorum*	No. of seedlings used in test	No. killed before emergence	No. killed after emergence	% infected roots in remaining seedlings	Total % roots infected in all seedlings
Isolations made after 1 week's decomposition of straws	1	10	9	0	71	97
	2	10	5	1	25	70
	3	10	3	1	47	68
	4	10	8	1	50	95
	5	10	10	0	0	100
Isolations made after 2 weeks' decomposition of straws	1	10	3	0	25	48
	2	10	7	0	57	87
	3	10	5	0	64	82
	4	10	5	1	71	88
	5	10	5	0	84	92
Isolations made after 3 weeks' decomposition of straws	1	10	1	0	8	17
	2	10	6	0	28	71
	3	10	6	1	85	96
	4	10	5	0	16	58
	5	10	4	2	64	85
Isolations from infected wheat roots	1	10	9	1	0	100
	2	10	7	3	0	100
	3	10	8	2	0	100
	4	10	8	2	0	100
	5	10	7	2	100	100
Type culture (Bennett's)	—	50	44	4	100	100

IV. Discussion

The observations reported have confirmed expectation by showing that *Fusarium culmorum* is an important primary colonizer of dead wheat straw buried in the soil. These results are a logical development of the observations of Samuel & Greaney (1937), and constitute proof of the ability of *F. culmorum* to live as a saprophyte in the soil. This fungus may now, therefore, be taken as belonging to Reinking & Manns' (1933, 1934) group of *soil inhabitants*, or *true soil fungi*. As a result of their extensive studies on the tropical soil *Fusaria*, these authors concluded that certain of the *Fusarium* spp., which occurred to an appreciable extent in all soils examined, were generally distributed in the soil, and must hence be considered as *soil-inhabiting fungi*, playing a part in the normal decomposition processes of the soil. Other of these tropical *Fusaria*, however, were isolated only locally, in past or present association with host plants. Such fungi dwindled and died out from the soil in the continued absence of suitable host plants, and were hence termed *soil invaders*. Garrett (1938) has suggested that the separation into these two ecological groups can be usefully applied to the whole group of root-infecting fungi. The *soil inhabitants* are considered to be primitive or unspecialized parasites with a wide host range, which are generally distributed throughout the soil; their parasitism is considered incidental to their saprophytic existence as members of the general soil microflora. The group of *soil invaders*, on the other hand, contains those more highly specialized parasites which are more or less closely associated with their host plants, and have little or no active saprophytic existence. Such restriction of saprophytic activity is considered to be due not so much to nutritional disabilities, as in the case of the true obligate parasites, as to the intense competition of all the saprophytic soil micro-organisms for existence upon decomposing plant remains in the soil.

These experiments have demonstrated the saprophytic capabilities of *F. culmorum*. They have further shown that this fungus is by no means confined to wheat or even to arable soils, since the soil types examined comprised two clay loams, one light sandy loam and a fen peat as arable soils, an allotment soil devoted to vegetable growing for some 70 years, and a glasshouse compost. Whilst only six soils have been examined in these two experiments, it seems highly probable that a wider search for *F. culmorum* with the aid of the straw technique employed in this study would establish its status as a regular fungus inhabitant of cultivated soils.

V. SUMMARY

A study has been made of the sequence of fungi developing on wheat straw buried in four arable soils, an allotment soil, and a glasshouse compost. Both natural untreated straw, and straw autoclaved in a 2 % solution of sodium nitrate were employed. *Fusarium culmorum* and *Mucor* spp. appeared to be dominant organisms in the earlier stages of straw colonization, but these were replaced by *Penicillium* spp. in the latter stages of decomposition. The nitrogenous treatment of the straw favoured the development of *Penicillium* spp. at the expense of *Fusarium culmorum* and *Mucor* spp. The pathogenicity of the *Fusarium culmorum* isolates to wheat seedlings was established by inoculation experiments.

The data provided by this investigation are considered sufficient to justify the inclusion of *F. culmorum* in Reinking & Manns' (1933) group of *soil-inhabiting Fusaria*, or true soil fungi.

It is a pleasure to express my indebtedness to Mr S. D. Garrett for suggesting this problem. I am also most grateful to him for his interest in the course of the investigation. My thanks are also due to Miss M. D. Glynne for helping me in the identification of some of the genera mentioned here, and to Miss L. Cunow, for help in carrying out the experiments. An acknowledgement is finally due to Dr F. T. Bennett, who originally supplied the type culture of *Fusarium culmorum*.

REFERENCES

BENNETT, F. T. (1928). On two species of *Fusarium*, *Fusarium culmorum* (W. G. Sm.) Sacc. and *Fusarium avenaceum* (Fries) Sacc. as parasites of cereals. *Ann. appl. Biol.* **15**, 213.

BLAIR, I. D. (1937). An investigation on footrot of wheat in New Zealand. *N.Z. J. Sci. Tech.* **19**, 1.

BROADFOOT, W. C. (1934). Studies on foot and root rot of wheat. IV. Effect of crop rotation and cultural practice on the relative prevalence of *Helminthosporium sativum* and *Fusarium* spp. as indicated by isolations from wheat plants. *Canad. J. Res.* **10**, 115.

FAWCETT, H. S. (1931). The importance of investigations on the effects of known mixtures of organisms. *Phytopathology*, **21**, 545.

FISHER, R. A. (1936). *Statistical Methods for Research Workers*. Sixth edition. Edinburgh.

GARRETT, S. D. (1934). Factors affecting the pathogenicity of cereal foot-rot fungi. *Biol. Rev.* **9**, 351.

—— (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667.

—— (1938). Soil conditions and the root-infecting fungi. *Biol. Rev.* **13**, 159.

Geach, W. L. (1933). Foot and root-rots of wheat in Australia. The influence of the combined action of *Fusarium culmorum* (W. G. Sm.) Sacc. and *Urocystis tritici* Koern. on the occurrence of seedling blight. *J. Coun. Sci. industr. Res. Aust.* **6**, 269.

Greaney, F. J. & Machacek, J. E. (1935). Studies on the control of root-rot diseases of cereals caused by *Fusarium culmorum* (W. G. Sm.) Sacc. and *Helminthosporium sativum* P., K and B. II. Pathogenicity of *H. sativum* as influenced by *Cephalothecium roseum* Corda in greenhouse pot tests. *Sci. Agric.* **15**, 377.

Hansen, H. N. (1929). Etiology of the pink-root disease of onions. *Phytopathology*, **19**, 691.

Hildebrand, A. A. (1934). Recent observations on strawberry root rot in the Niagara Peninsula. *Canad. J. Res.* **11**, 18.

Hildebrand, A. A. & Koch, L. W. (1936). A microscopical study of infection of the roots of strawberry and tobacco seedlings by micro-organisms of the soil. *Canad. J. Res.* **14**, 11.

Koch, L. W. (1935). Recent investigations on tobacco root rot in Canada. *Canad. J. Res.* **13**, 174.

Reinking, O. A. (1934). Parasitic and other *Fusaria* counted in Costa Rica and Panama soils. *Zbl. Bakt.* Abt. 2, **90**, 4.

—— (1935). Soil and *Fusarium* diseases. *Zbl. Bakt.* **91**, 243.

Reinking, O. A. & Manns, M. M. (1933). Parasitic and other *Fusaria* counted in tropical soils. *Z. Parasitenk.* **6**, 23.

—— —— (1934). Parasitic and other *Fusaria* counted in Colombia soils. *Zbl. Bakt.* Abt. 2, **89**, 502.

Russell, T. A. (1932). Observations on foot rot diseases of cereals. *Trans. Brit. mycol. Soc.* **16**, 253.

Samuel, G. & Greaney, F. J. (1937). Some observations on the occurrence of *Fusarium culmorum* on wheat. *Trans. Brit. mycol. Soc.* **21**, 114.

Simmonds, P. M. (1928). Seedling blight and footrot of oats caused by *Fusarium culmorum*. *Bull. Dep. Agric. Can.* **105** (N.S.).

Tu, C. (1929). Physiologic specialization in *Fusarium* spp. causing head blight of small grains. *Phytopathology*, **19**, 143.

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AN ACCOUNT OF THE DEVELOPMENTAL STAGES OF SOME APHIDOPHAGOUS SYRPHIDAE (DIPT.) AND THEIR PARASITES (HYMENOPT.)

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(With Plates XXV–XXX)

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The developmental stages of some aphidophagous Syrphidae

A general description of the larvae and pupae of Syrphidae is given by Metcalf in his paper on the Syrphidae of Maine (1916, pp. 202–5). He separates the larvae and pupae into five "types" which differ according to the food and habits of the larva, and a comprehensive key is given for the identification of these types (Metcalf, 1916, pp. 207–12, 256). In this work, Metcalf describes the distinguishing features of the aphidophagous type of Syrphid larva, which are as follows: "Body subcylindrical, flattened ventrally, and much attenuated anteriorly. Posterior respiratory process short, the spiracles either straight or convoluted, never denticulated. Interspiracular ornamentation consisting of bare hairs, nodules, ridges or lamellae, never of plumose hairs. Mouth parts of two ∧-shaped jaws. Segmental hairs single. If prolegs present, without specialized vestiture. Anterior pupal respiratory cornua apparently wanting."

The following descriptions are chiefly concerned with the pupae of additional species of aphidophagous Syrphidae which I have examined. A detailed description of the larva is not given, since the larva may be identified by the nature of the posterior respiratory trunk, which is unaltered in the pupal stage. A key has been constructed for the identification of the pupae, using my own observations, and incorporating Metcalf's descriptions of certain species. In describing the pupae, I have

used the terms employed by Metcalf; he gives a glossary of these (1916, pp. 250–61).

When known, the stage of development at which hibernation occurs in different species has been noted.

Eggs which were laid by identified Syrphidae in captivity were examined, and found to show interesting differences in the sculpturing of the chorion. Examination of the eggs of a few species only was possible, and descriptions of these are included with those of the larvae and pupae.

The sculpturing of the chorion of the eggs of Syrphidae is characteristic. The basic pattern (Metcalf, 1916, p. 202) consists of rows of elongated elevations, separated by depressed areas. These rows run more or less longitudinally, and the elevations of one row tend to alternate with those of the rows on either side. This fundamental pattern is complicated in various ways, and the ultimate pattern of the chorion is characteristic of the species, with the exception of very closely allied species, such as *Syrphus luniger* Mg. and *S. corollae* Fab., and again *S. ribesii* (L.) and *S. vitripennis* Mg. When the sculpturing of the free upper surface is complicated, it is usually different from that of the lower surface, which remains simple.

(1) and (2) *S. luniger* Mg. and *S. corollae* Fab.

I have been unable to separate the eggs, larvae and pupae of these species; separate descriptions are therefore not given.

Eggs. Although indistinguishable from one another, the eggs of these species are readily distinguished from those of other Syrphidae examined. The lower surface of the chorion is made up of relatively wide, elongated and slightly elevated areas (4–10 times as long as broad), separated by narrow, nearly parallel-sided depressions. The latter are transversed by a few short branches which connect the elevated areas (Pl. XXV, fig. 8). On the upper surface, the elevated areas are narrowed, the transverse branches of the network are more numerous, and as wide as the longitudinal ones, while the depressed areas are considerably wider. Thus, the basic undulating network is evident. The distinguishing features of the eggs of these species are the elongated "crests" on the upper surface, which coincide with the regularly alternating rows of short, narrow elevations of the chorion (Pl. XXV, figs. 6, 7).

Larva. The newly hatched larva is yellowish grey in colour, but as it grows it rapidly assumes the dark grey colour of the full-grown larva. The latter is about 1·5 cm. in length, and the transverse rows of segmental spines are prominent.

Pupa (Pl. XXV, figs. 1, 2). The dimensions of the pupa are as follows: length 7–7·3 mm. (average 7·16 mm.); width 3·6–3·9 mm. (average 3·75 mm.); height 3·0–3·4 mm. (average 3·27 mm.). A number of "starvation dwarf" pupae were formed in the laboratory, when the larvae were not given a sufficient supply of aphids, and pupated before they were fully grown. The dimensions of these pupae were: length 4·5–5·5 mm. (average 5·0 mm.), width 2·7–3·0 mm. (average 2·8 mm.), height 2·1–

2·8 mm. (average 2·37 mm.). These small pupae have not been found in nature, but they may occur when the larva is unable to find an adequate supply of food—for example, in late September and early October when aphids are scarce.

These species hibernate as pupae. The pupae are well elevated throughout their length, so that the dorsal line is convex, tapering rather abruptly to the posterior respiratory tube. Until the adult emerges, the pupa is purplish black in colour, but the colour of the empty puparium varies from a brownish black to a dark grey-black. The black colour is due partly to the pigment in the puparium, and partly to the presence of short, black integumental spines. These spines vary in size and density of distribution, being larger and more numerous in certain well-defined areas. Thus, a thin, black mid-dorsal line is evident, and immediately lateral to this are five or six pairs of oblique dark patches, which give the pupa a characteristic appearance (Pl. XXV, fig. 2). Segmental spines, so prominent in the larva, are small, and light in colour.

The posterior respiratory tube is short (about half as long as broad), dark brown in colour, and shining. The three pairs of spiracular openings are borne on well-elevated black carinae. The dorsal openings are shorter, and more elevated than the lateral and ventral pairs, which are elongated, and lie close together on the ventral side. The "buttons" or circular plates are well developed, and situated dorsally. A conspicuous, short sharp dorsal spiracular spine is developed on the median side of each spiracular plate. The interspiracular ornamentation consists of small, inconspicuous nodules (Pl. XXV, figs. 3–5).

(3) and (4) *S. ribesii* (L.) and *S. vitripennis* Mg.

The larvae and pupae of these two species are indistinguishable. The eggs of *S. ribesii* only were examined.

Eggs. The eggs of *S. ribesii* are laid singly or in groups, and while the chorion on the lower surface is simple and similar to that of the eggs of *S. lumiger* described above, on the upper surface it shows a complicated sculpturing. Again the typical basic pattern is seen, but the elevations are short and broad, and end in large, shallow cup-like structures, which are very characteristic, and may be seen with the naked eye (Pl. XXV, figs. 9, 10).

Larva. The larva grows to a comparatively large size (about 2 cm.) and is a pale orange yellow colour. These species hibernate in the larval stage, pupating in early spring.

Pupa. The pupa is light brown in colour, and bare except for a few pale segmental spines. Some indication of the larval segmentation persists in the form of transverse lines, which tend to lie together in groups of three (Pl. XXVI, fig. 1). The pupa is well elevated throughout its length, the dorsal line being convex, and almost semicircular. Normal pupae are large, measuring 7·3–7·8 mm. in length (average 7·4), 3·9–4·7 mm. in width (average 4·1) and 3·5–4·2 mm. in height (average 3·85). As with *S. lumiger*, a number of "starvation dwarfs" of *S. ribesii* were bred in the laboratory. These pupae measured 5·2–6·5 mm. in length (average 6·2 mm.), 3–3·7 mm. in width (average 3·1 mm.) and 2·9–3·5 mm. in height (average 3·1 mm.).

The posterior respiratory trunk is conspicuous, since it is darker in colour than the integument, and hard and shining. It is a little broader than long. The spiracles are not elevated, but are long and straight, and extend well down the sides of the tube.

The relatively wide apertures are bounded by narrow, thickened black edges. The circular plates are well developed, and have well developed dorso-ventral ridges on the median side. Conspicuous nodules, elevated above the spiracles from the interspiracular ornamentation.

(5) *S. torvus* O.-S. (Pl. XXVI, fig. 2)

The pupa of this species has been described and illustrated by Metcalf (1916, pp. 240–1, fig. 33, nos. 13–15), but the following observations may be of use in identification. In the specimen I examined, the nodules forming the interspiracular ornamentation were similar to those seen in *S. ribesii*, being well developed, and raised above the spiracles, whereas Metcalf describes them as "inconspicuous ridges". The pupa of this species is marked by transverse lines which are placed at regular intervals throughout its length. This feature serves to distinguish it from the pupae of *S. ribesii* and *S. vitripennis*, in which the transverse lines are not so well marked, and are arranged in groups of three.

(6) *S. auricollis* Mg.

Eggs and two puparia of this species were examined, but the larva was not available.

Eggs. These are laid singly, or in groups, but are not arranged in any definite manner. The sculpturing of the chorion is simple, being in the form of a fine undulating longitudinal transverse raised network. The longitudinal areas are elevated, and are joined by short transverse branches which traverse the intervening depressions (Pl. XXVI, fig. 7).

Pupa (Pl. XXVI, figs. 3, 4). The measurements of the puparia examined were: length 6·0 and 6·1 mm., width 3·2 and 3·4 mm., height 2·8 and 2·7 mm. respectively. The pupa is well elevated anteriorly, and tapers from about the middle to the posterior end. The integument is smooth and shining, with no segmental or integumental spines. The puparia examined differed considerably in colour, one being very light, while the other was deeply pigmented with black. In spite of this, the black, mid-dorsal markings were present in both specimens, and appear to be characteristic of the species. At least three anterior markings are present, and two small posterior ones visible in the deeply pigmented puparium. The anterior markings have the appearance of wide, shallow goblets, with dense black centres and bases, and sides of a lighter grey. The markings situated posteriorly are small, the central parts being black and V-shaped; while the "sides" have become separated and are situated dorso-laterally. These markings of the pupa appear to be correlated with the segmentation of the larva.

The posterior respiratory tube (Pl. XXVI, figs. 5, 6) is about as long as broad, and shows a well-marked constriction near the base. The spiracles are slightly elevated, the dorsal pair of openings more so than the lateral and ventral pairs. The lateral openings lie nearer to the ventral than to the dorsal pair. The circular plates are easily seen, and slightly elevated ridges are present on the median side. The interspiracular ornamentation consists of small, inconspicuous nodules.

(7) *S. cinctellus* (Zett)

Only one puparium of this species was examined, but it is quite distinct. The following measurements were taken: length 6·7 mm., width 3·6 mm., height 2·5 mm. The pupa is well elevated anteriorly, and the dorsal line is convex in this region

(Pl. XXVI, fig. 8). As the pupa tapers to the posterior end this line becomes concave, a feature which is accentuated by the upward turning of the ventral line posteriorly. The integument is light in colour, without markings or spines.

The posterior respiratory tube (Pl. XXVI, figs. 9, 10; Pl. XXVII, fig. 1) differs markedly from that of any other Syrphid examined. Dorsally the "wall" of the tube is about half as long as broad, but ventrally it is extremely short, almost absent. A well-defined ridge surrounds the base of the tube, forming a flattened "shelf". The dorsal spiracular openings are well elevated, but the other two pairs lie flat on the stigmal plates, close together on the ventral side. The "circular plates" are not rounded or elliptical as in other Syrphid pupae, but are rather rectangular, and elongated dorso-ventrally. On the median sides of each of these plates is a well-elevated dorso-ventral ridge, which terminates in a narrow carina. The interspiracular ornamentation consists of small, inconspicuous nodules.

(8) *S. albostriatus* (Fln.)

One larva, and one puparium of this species were seen.

Larva. The larva is rather flattened, dark grey in colour with a granulated appearance. The most striking features of the larva are the projections of the integument. These are regularly arranged, three to each segment on each side, and an elongated posterior pair. These projections increase the width, and so accentuate the flattened appearance. The projections end in well-developed spines, and the mid-dorsal pairs of segmental spines are also conspicuous.

Pupa (Pl. XXVII, figs. 2, 3). Like the larva, the pupa is dark grey in colour, and has numerous transverse indentations, set close together, which are due to the shrinking of the larval integument. The lateral projections seen in the larva are present, but the regularity of size and arrangement is lost. Unlike the larva, the pupa is not flattened, as the following measurements show. Length 7·5 mm., width 3·3 mm., height 2·5 mm.

The dorsal line is convex anteriorly, but becomes concave after the middle; the ventral line is slightly arched in front, and then turns upwards rather sharply in the posterior region. There are no integumental spines present, and segmental spines are also absent, with the exception of the mid-dorsal pairs. These are very conspicuous, for they are slightly raised, and situated in light-coloured areas, which are bounded by oblique, slightly darkened dorso-lateral bands.

The posterior respiratory tube is very conspicuous, being more than twice as long as broad, black, shining and pointed. The two spiracles are widely separated distally by a deep indentation. The great development of the stigmal plates on the median edges to form large ridges accentuates this median depression, and gives the whole structure a pointed appearance. As a result of this, the spiracles which are situated on well-elevated carinae, lie laterally, near the end of the tube, instead of in the usual terminal position. Circular plates were not distinctly seen, and the interspiracular ornamentation is restricted to small ridges which are seen when the tube is viewed from above (Pl. XXVII, figs. 4–6).

(9) *S. balteatus* (Deg.)

The larvae of this species are found abundantly on cabbage, and a large number of larvae and pupae were examined.

Larva. When fully grown, the larva is large, measuring about 2 cm. in length. It is light green (often pink-tinged) in colour, with the white dorsal lines well developed.

Pupa (Pl. XXVII, figs. 7, 8). Length 6·7–7·8 mm. (average 7·7), width 2·9–4 mm. (average 3·4), height 2·8–3·8 mm. (average 3·2). The pupa is well elevated in the anterior half, and the dorsal line is broadly convex in this region. After the middle, the pupa tapers rather suddenly, so that the dorsal line becomes concave. The ventral line is usually fairly straight. Integumental and segmental spines are absent, and the surface is smooth and shining. The colour of the puparium varies considerably, some being almost white, with hardly any markings, while others are dark brownish grey, and have deeply pigmented markings on the dorsal surface. When present, these markings are characteristic, and in the empty puparium consist of two wide curved transverse bands situated anteriorly. These markings correspond to the segmentation of the larva, as do the pairs of small, elongated dark-coloured areas which lie laterally to them. The latter are found in the posterior region also, although the larger markings are absent.

The posterior respiratory trunk is broader than long, and shows a well-marked constriction about the middle of its length. The spiracles are not elevated, and the wide apertures have narrow black edges. They are long and straight, about equidistant from one another, and extend well down the sides of the tube. The circular plates are readily distinguishable. Dorsal spiracular spines or ridges are absent, and the interspiracular ornamentation is in the form of elongated nodules (Pl. XXVII, figs. 9, 10).

Catabomba O.-S.

Puparia of the two species of *Catabomba* were examined, and found to differ strongly in the characters of the posterior respiratory trunk. This is of special interest, since the adults are very similar.

(10) *C. pyrastri* (L.) (Pl. XXVIII, fig. 5)

Larva. The larvae are green with a very distinct white dorsal stripe.

Pupa. Nine puparia of this species were examined, and found to have the following dimensions: length 7·0–8·2 mm. (average 7·9), width 3·4–4·5 mm. (average 4·4 mm.), height 3·2–4·2 mm. (average 3·7 mm.). The puparium is well elevated throughout its length, the dorsal line being almost semicircular. The integument is light brown in colour, and is covered with fine spines, which are visible only with the aid of a binocular microscope. The majority of these are light in colour, but near the posterior end they are larger, darker and more numerous than elsewhere. Small, light-coloured segmental spines are also present. The pupa is marked by transverse lines which tend to be arranged in pairs, and appear to indicate the larval segmentation.

The posterior respiratory tube is very short, being about four times as broad as long; it is dark brown in colour, and has the appearance of being sunk in the integument. A well-marked indentation separates the stigmal plates. The apertures are borne on well-elevated black, shining carinae, the dorsal pair being more elevated than the lateral and ventral pairs, which lie close together on the ventral side. The circular plates are well developed, and no dorsal-spiracular ridge or spine is present. The interspiracular ornamentation is in the form of small, but distinct black nodules (Pl. XXVIII, figs. 6–8).

(11) *C. selenitica* (Mg.) (Pl. XXVIII, fig. 1)

Two puparia of this species were examined. The puparium is very similar to that of *C. pyrastri*, but is larger, having an average length of 8·8 mm., width 5·0 mm., and height 4·7 mm. It is light brown in colour and is covered with very small, light-coloured, integumental spines. As in *C. pyrastri*, these spines are larger, darker, and more numerous posteriorly. A few light-coloured segmental spines are also present. Transverse markings of the integument are visible, but do not appear to be arranged in any definite manner.

The posterior respiratory tube (Pl. XXVIII, figs. 2–4) is short, being less than half as long as broad; it is brown and shining. The spiracular apertures are long and straight, equidistant from one another, and slightly elevated on black carinae. The dorsal apertures are shorter, and slightly more elevated than the lateral and ventral pairs. The circular plates are well developed, and a pair of conspicuous dorsal spiracular ridges are present. Minute black nodules constitute the interspiracular ornamentation.

(12) *Baccha elongata* (Fab.)

Only one puparium of this species was examined, but it is very distinctive. The pupa was formed in early spring, the species hibernating in the larval stage.

Pupa (Pl. XXVIII, figs. 9, 11). The dorsal line is broadly convex anteriorly, but it becomes concave before the middle, so that the anterior part is rather bulbous in appearance. This is accentuated by the broad flat shape of the posterior region. The ventral line follows the general shape of the dorsal one, being convex anteriorly and concave posteriorly. The pupa is elongated, the ratio of length to width and height being greater than in any Syrphid pupa of the aphidophagous type examined. Length 6·3 mm., width 2·0 mm., height 2·0 mm.

The integument is smooth and bare, both integumental and segmental spines being absent. The general colour is dark brown, but a characteristic pattern is formed by intersecting areas of a light colour. A transverse oval-shaped light-coloured area is situated mid-dorsally in the anterior region, and behind this are two pairs of wedge-shaped areas, also light in colour, but laterally placed. Extending from before the middle to the posterior end are dark-coloured areas, separated by corresponding areas of a light colour. These latter lie in the mid-dorsal, latero-dorsal, and lateral regions (Pl. XXVIII, fig. 11).

The posterior respiratory tube is brown in colour, slightly longer than broad, and situated well in front of the extremely flattened posterior edge. The stigmal plates, separated by a slight median indentation, bear the spiracular apertures which are situated on conspicuous, slightly elevated black carinae; they lie close together, but are quite distinct. The circular plates are well developed, but there is no interspiracular ornamentation, and dorsal spiracular spines or ridges are absent (Pl. XXVIII, fig. 10).

Platychirus Lep.

Eggs (Pl. XXIX, fig. 3). The eggs of *P. albimanus* (Fab.) have been examined, and found to differ from those of species of *Syrphus* in the absence of the fine raised network characteristic of the chorion of the upper surface. The ornamentation of the chorion consists of large, irregular elongated areas, which are elevated, and tend to lie in alternating rows. These are separated by narrow depressions, and a few short,

raised transverse branches traverse these, and connect the elevated areas. These areas in turn bear a large number of small circular elevations which are very numerous near the irregular projections at the edges, and give the chorion a characteristic appearance.

Larva. The larvae of species of *Platychirus* are distinguished by the purplish green colour, and the V-shaped markings on the dorsal surface. Like the puparia, the larvae do not appear to have distinguishing specific characters, but the genus is readily recognized. Hibernation occurs in the larval stage.

Pupae of *P. scutatus* (Mg.) *P. albimanus* (Fab.), *P. clypeatus* (Mg.) and *P. scambus* Staeg. have been examined, but it has not been found possible to distinguish the species.

(13) *Platychirus scutatus* (Mg.) (Pl. XXIX, fig. 1)

Pupa. The pupa is small, the measurements being: length 4·9–5·8 mm. (average 5·3 mm.), width 2·2–2·8 mm. (average 2·6 mm.) and height 2·1–2·6 mm. (average 2·3 mm.). The dorsal line is convex throughout, and the ventral line slightly so in the posterior part. The integument is light in colour, shining and without integumental or segmental spines, but is marked by a large number of transverse lines.

The posterior respiratory tube (Pl. XXIX, fig. 2) is terminal in position, and is about half as long as broad. The apertures are very small and slit-like, and the three present on each stigmal plate lie together in a black, slightly elevated "carinal plate". The circular plates are well developed; dorsal spiracular spines or ridges are absent. The interspiracular ornamentation is present in the form of minute black nodules, situated on the "carinal plates" which bear the apertures.

(14) *Sphaerophoria scripta* (L.)

The larva of this species is very common and may be found in winter, for hibernation takes place in this stage. It is 5–7 mm. long, and is yellowish green in colour, with a pair of white dorso-lateral lines, formed by the underlying fat.

Pupa (Pl. XXIX, figs. 4, 5). Fifty-two puparia were examined, and found to have the following measurements: length 5·2–6·9 mm. (average 5·7 mm.), width 2·1–3·1 mm. (average 2·6 mm.), height 2·0–3·1 mm. (average 2·5 mm.). The pupae are elongated in appearance, and the dorsal line is convex anteriorly, sloping gradually to the posterior end. The ventral line is usually straight, but may be slightly convex. The integument is generally light in colour, but some of the puparia examined were pigmented, though no characteristic colour markings were formed. Integumental and segmental spines are absent.

The posterior respiratory trunk is conspicuous, for it is about twice as long as broad, and shows a well-marked constriction about the middle of its length. The component tracheae are separated distally by a deep indentation, but they are not divergent, the sides being parallel throughout. The stigmatal apertures are wide, and are borne on conspicuous black carinae which are very slightly elevated. The circular plates are well developed, but dorsal spiracular spines or ridges, and interspiracular ornamentation are absent (Pl. XXIX, figs. 6, 7).

(15) *Sphaerophoria flavicauda* Zett. (Pl. XXIX, fig. 8)

The puparium of this species is very similar to that of *Sphaerophoria scripta*, but may be identified by its size and shape. It is small, the measurements being: length 4·5–5·2 mm. (average 4·9 mm.), width 1·9–2·4 mm. (average 2·1 mm.), height 1·8–

2·1 mm. (average 2·0 mm.). As a rule, the ventral line turns sharply upwards in the posterior two-fifths, giving the puparium a characteristic appearance.

A key has been compiled for the identification of the pupae described here, and incorporating the following aphidophagous species, described by Metcalf:

(1) *Didea fuscipes* Loew. (1911, pp. 339–40, Pl. XVI, figs. 5–7).
(2) *Syrphus torvus* O.-S. (1911, p. 342, Pl. XVII, figs. 13–15).
(3) *Paragus bicolor* (Fab.) (1911, p. 400, Pl. XIX, figs. 25–28).
(4) *Paragus tibialis* (Fln.) (1911, pp. 402–3, Pl. XIX, figs. 33–36).
(5) *Syrphus americanus* Wied. (1912, pp. 484–5, Pl. XXIII, figs. 49, 50, 55, 56).
(6) *Allograpta obliqua* (Say) (1912, pp. 536–7, Pl. XXX, figs. 68–70).
(7) *Sphaerophoria cylindrica* (Say) (1912, p. 540, Pl. XXX, figs. 72, 73, 78).
(8) *Syrphus xanthostoma* Williston. (1913, pp. 82–3, Pl. IV, figs. 84–87).
(9) *Melanostoma mellinum* (L.) (1916, p. 230, fig. 30, nos. 5, 7, 9).
(10) *Syrphus nitens* (Zett.) (1916, p. 244, fig. 34, nos. 3, 4, 6).
(11) *Pipiza pisticoides* Williston (1916, p. 226, fig. 29, nos. 2, 3).
(12) *Xanthogramma divisa* Williston (1917, p. 160, fig. 9 B, fig. 8 E, G, I, J).
(13) *Syrphus oronoensis* Metcalf (1917, p. 164, fig. 10 B, D, E, F, G).
(14) *Platychirus perpallidus* Verrall (1916, p. 170, fig. 11 H, I, J).
(15) *Syrphus knabi* Shannon (1917, p. 175, fig. 12 D, E, F, G).

Metcalf (1916) gives a key for the separation of "types" of Syrphid larvae and pupae. These various types are distinguished according to the food and habitat of the larvae, and so are designated "Filth-inhabiting type", "Boring type", "Aphidophagous type", etc. A description of the latter has been given at the beginning of the present paper.

KEY FOR THE IDENTIFICATION OF SOME SYRPHID PUPAE

1. *a.* Pupa tapering from about the middle to the posterior end (e.g. Pl. XXVII, fig. 8) **2**
   b. Pupa not tapering, the dorsal line being convex,[1] and the curve from the anterior to the posterior ends almost semicircular (e.g. Pl. XXV, fig. 1) **12**

2. *a.* Pupa tapering rather suddenly after the middle, so that the dorsal line is at first convex and then concave **3**
   b. Pupa tapering gradually towards the posterior spiracular trunk ... **7**

3. *a.* Pupa smooth, and without marginal serrations **4**
   b. Pupa not smooth, marginal serrations present **6**

4. *a.* Pupa three or more than three times as long as broad or as "high".
   b. Pupa two or two and a half times as long as broad or as "high". Usually two or three dark transverse markings on the antero-dorsal surface. Posterior spiracular trunk short, with rather straight, non-elevated spiracles (Pl. XXVII, figs. 7–10). **Syrphus balteatus** (Deg.).

5. *a.* Pupa with short posterior spiracular trunk; spiracles arranged close together and not elevated. The markings on the antero-dorsal surface are characteristic (Pl. XXVIII, figs. 9–11). **Baccha elongata** (Fab.).

[1] In *Syrphus auricollis* Mg. the pupa is only slightly convex, and may be confused with (*a*).

5. *b.* Pupa with the posterior spiracular trunk elongated, and having a characteristic constriction two-thirds the distance from the base. Slit-like spiracles, moderately elevated and short. Prominent dorsal-spiracular ridge, rather crescent-shaped. Interspiracular ornamentation consists of ridges bearing single small whitish setae.

Syrphus oronoensis Metcalf.

6. *a.* Pupa with marginal serrations arranged in groups of three. Six or seven prominent transverse blackish bands mark the integument. Posterior end truncated. Posterior spiracular trunk elongated with the stigmatal plates separated by a shallow depression. Dorsal spiracular spine inconspicuous, but interspiracular ridges broadly carinate and rugose. **Xanthogramma divisa** Will.

b. Pupa with marginal serrations not regularly arranged in groups of three. No definite colour markings, but with prominent dorsal setae. Posterior end elongated. Posterior spiracular trunk elongated, appearing to be pointed. Dorsal spiracular ridge very well developed, and the apertures lie on the sides of the tube (Pl. XXVII, figs. 2–6).

Syrphus albostriatus (Fln.)

7. *a.* Pupa smooth **8**
b. Pupa not smooth, but with well-developed segmental spines. No marginal serrations. Posterior spiracular trunk elongated. Dorsal spine well marked. **Paragus tibialis** (Fln.)

8. *a.* Posterior respiratory trunk very short—less than half as long as broad **9**
b. Posterior respiratory trunk elongated—at least twice as long as broad **10**

9. *a.* Posterior respiratory trunk of usual form, with flattened distal surface. Length of tube equal all round. No dorsal spiracular ridge or spine.

Melanostoma mellinum (L.)

b. Posterior respiratory trunk much longer anteriorly than posteriorly, and having a ridge around the base. Spiracular plates elongated and parallel sided. Dorsal spiracular ridge well developed, carinate. Dorsal spiracles well elevated and separated from the others which lie flat (Pl. XXVI, figs. 8–10 and Pl. XXVII, fig. 1). **Syrphus cinctellus** (Zett.)

10. *a.* Posterior spiracular trunk with short but distinct spur-like nodule on each interspiracular space, which is continued down the side of the tube as a more or less evident ridge. **Allograpta obliqua** (Say).

b. Posterior spiracular trunk without interspiracular spines **11**

Sphaerophoria sp.

11. *a.* Pupa small (average length 4·9 mm.) with characteristic elevation of the postero-ventral line (Pl. XXIX, fig. 8).

Sphaerophoria flavicauda Zett.

b. Pupa larger (average length 5·8 mm.) without marked elevation of the postero-ventral line (Pl. XXIX, figs. 4–7).

Sphaerophoria scripta (L.) and **Sphaerophoria cylindrica** (Say)

12. *a*. Pupa smooth, usually light in colour **13**
b. Pupa spiny, usually dark in colour **19**

13. *a*. Pupa small (4·5–5·5 mm. long, 1·5–2 mm. wide, 1·5–2 mm. high). Posterior spiracular trunk short with the spiracles slit-like and situated on a black, slightly elevated "plate". No interspiracular spines or ridges (Pl. XXIX, figs. 1, 2). **Platychirus** spp.
b. Pupa larger in all dimensions. Posterior respiratory trunk long, but not particularly so; the spiracles well developed, separate. Interspiracular ornamentation present, and also dorsal spine or ridge **14**

14. *a*. Posterior, slit-like spiracles greatly elongated and very sinuous, the dorsal one much elevated. Interspiracular ornamentation consists of inconspicuous nodules. **Syrphus nitens** (Zett.)
b. Posterior spiracles straight or curved, but never sinuous **15**

15. *a*. Pupa with characteristic colour markings. Dorsal line not broadly convex. Interspiracular ornamentation in the form of small, carinate knobs (Pl. XXVI, figs. 3–6). **Syrphus auricollis** Mg.
b. Pupa without definite markings. Dorsal line almost semicircular. Interspiracular ornamentation consists of well-elevated ridges or nodules ... **16**

16. *a*. Posterior respiratory tube as long, or slightly longer than broad ... **17**
b. Posterior respiratory tube about one-third broader than long ... **18**

17. *a*. Puparium exceptionally inflated dorsally, the posterior inflation being equal to, or greater than that anteriorly. Interspiracular spines short, blunt, spur-like, but prominent, standing well above the spiracular openings. Dorsal spiracular spine short. **Syrphus xanthostoma** Will.
b. Puparium not exceptionally inflated. Interspiracular ornamentation is of short, sharp, well-elevated carinae. Well-developed dorsal ridge. **Syrphus knabi** Shannon

18. *a*. Pupa with transverse markings arranged at regular intervals, not in groups (Pl. XXVI, fig. 2). **Syrphus torvus** O.-S.
b. Pupa with transverse markings light, irregular, with a distinct tendency to be arranged in groups of three or four, apparently marking the larval segmentation (Pl. XXVI, fig. 1). **Syrphus ribesii** (L.) and **S. vitripennis** Mg.

19. *a*. "Segmental and integumental spines not visible to the naked eye, but give the pupa a 'spinulose' appearance when seen under the lower power of the microscope" (Metcalf). Spines light in colour. General colour a light testaceous brown **20**
b. Segmental spines plainly visible; light or dark in colour. Integumental spines are short, sharp and black in colour when present **22**

20. *a*. Pupa small (4–5 mm. long); the posterior spiracular process elongated (about twice as long as broad), and having a definite constriction about the middle. Interspiracular spaces with long, slender hairs. **Pipiza pisticoides** Will.

b. Pupa large (7–9 mm. long). Posterior spiracular trunk one-third or less than one-third as long as broad. Interspiracular spaces with small, inconspicuous nodules **21**

Catabomba spp.

21. *a*. Posterior spiracles well elevated on broad black carinae, the dorsal spiracles more elevated than the others. Dorsal spiracular spine or ridge absent (Pl. XXVIII, figs. 5–8). **Catabomba pyrastri** (L.)

b. Posterior spiracles narrow, slightly elevated. Dorsal spiracular ridge present (Pl. XXVIII, figs. 1–4). **Catabomba selenitica** (Mg.)

22. *a*. Integumental spines present, black **23**

b. Integumental spines absent. Segmental spines present, and arranged in a definite order. Posterior spiracular trunk very slightly longer than broad. Interspiracular spaces with no visible spines, but the dorsal spiracular ridge is well developed. **Paragus bicolor** (Fab.)

23. *a*. Segmental spines black, conspicuous, and "crown the summits of conspicuous conical elevations". Posterior respiratory tube hard, black, with elevated stigmata. Interspiracular ornamentation of thin, laminate ridges, as high as the stigmata, two to each space; they run down the side of the tube, which is short. **Didea fuscipes** Loew.

b. Segmental spines light in colour, inconspicuous. Posterior spiracular trunk short, with well-elevated stigmata, the lateral and ventral pairs close together. Dorsal spiracular spine well developed, sharp. Interspiracular ornamentation of inconspicuous nodules **24**

24. *a*. Integumental spines evenly distributed. **Syrphus americanus** Wied.

b. Integumental spines larger and darker in patches, there being several dorso-lateral pairs of these (Pl. XXV, figs. 1–5).

Syrphus luniger Mg. and **Syrphus corollae** Fab.

Some parasites of Syrphidae

Syrphidae are not subject to a very high percentage parasitism, but during the course of this work several hymenopterous parasites have been bred. In the following account, an attempt has been made to arrive at some conclusions regarding the biology of these parasites from records kept by Dr O. W. Richards of the Imperial College of Science and Technology and myself. Dr Richards has kept records of any Syrphid larvae and pupae he came across while engaged in his own work during the years 1932–6 and I am indebted to him for the use of these records. All specimens were obtained at Slough, Bucks., and all Dr Richards' were collected on cabbage, where they were feeding on *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulz.).

HYMENOPTEROUS PARASITES BRED FROM SYRPHID PUPAE

(1) ICHNEUMONOIDEA.

(*a*) BASSINAE.

Bassus laetatorius (Fabr.).
B. annulatus Grav.
Homocidus signatus (Grav.).
H. tarsatorius (Panz.).
H. nigritarsis (Grav.).
H. pictus (Grav.).
Promethus monticola Sey.
P. sulcator (Grav.).

(*b*) CRYPTINAE.

Gelis sp.

(2) CHALCIDOIDEA.

(*a*) ENCYRTIDAE.

Syrphophagus aeruginosus (Dalm.).

(*b*) PTEROMALIDAE.

Pachyneuron formosum Walker.

(3) PROCTOTRUPOIDEA (Calliceratidae).

Lygocerus sp.
Trichosteresis försteri Kieff.
Conostigmus sp.

(1) *Bassus*

With the exception of 1 ♂ *Bassus annulatus* Gr. which was bred from the puparium of *Syrphus luniger*, only ♀♀ of *Bassus laetatorius* (Fab.) have been bred.

A pupa of *Syrphus luniger* was collected on 11 September 1933, and on 2 February 1934 a ♂ *Bassus annulatus* emerged. This is early in the year for the emergence of the parasite, but the puparium was kept indoors during the winter. Twenty-six ♀♀ of *B. laetatorius* were bred, the hosts being *Syrphus luniger*, *S. balteatus* and *Sphaerophoria scripta*.

The records show that there are at least two generations of this parasite per annum, and there is some indication of the existence of one or two more. The parasite hibernates in the body of the larva of *Sph. scripta* and *Syrphus balteatus*; for these Syrphidae do not pupate until the

spring, while it is a parasite of the puparium of *S. luniger* during the winter, since hibernation occurs in the pupal stage in that species.

The first generation of *Bassus laetatorius* emerges in spring (March–May) and the last in the autumn (September and October). This is clearly shown by the records kept. Little or no collecting was done during July and August, except in 1935, but the records for that year show that individuals of this species emerge during these months, and suggest that at least one generation of the parasite emerges about this time. Morley (1911) says that this Ichneumonid "is abundant from the end of July to the beginning of September", an observation which supports the suggestion that one or more generations emerge at this time of the year.

Three females which emerged in May 1935 were put in tubes containing eggs of *Syrphus luniger*, *S. ribesii* and *Melanostoma mellinum* respectively. A cut and moistened raisin was put in for food. In each case the ♀♀ pierced the Syrphid eggs present, and a ♀ *Bassus laetatorius* emerged from one of the pupae of *Melanostoma mellinum*, which developed from these eggs. The duration of the pre-adult period was about five weeks.

Again, a ♀ *Bassus laetatorius* was put in a tube containing eggs and first-stage larvae of *Syrphus luniger*. The parasite on this occasion proceeded to eat the eggs, using the young larvae for oviposition. Later, a ♀ *Bassus laetatorius* emerged from a puparium which developed from one of these larvae. Parthenogenesis is the rule in this species, and oviposition occurs soon after the emergence of the adult parasite. The parasite will live for a considerable time in tubes containing a cut, wet raisin. In the laboratory, under these conditions, I have found the parasite to live as long as three weeks.

According to the records available, *Syrphus balteatus* and *Sphaerophoria scripta* are the chief hosts of this parasite at Slough, but it has been bred from pupae of *Syrphus luniger*, *Platychirus* sp. and *Melanostoma mellinum*, and has been observed to attack the eggs of *Syrphus ribesii*. In this instance, the records may be misleading, for larvae and pupae of *S. balteatus* and *Sphaerophoria scripta* are found in far greater numbers than the others on cabbages, where most of the collecting was done.

The percentage parasitism of *Syrphus balteatus* by this Ichneumonid was 3·0, and 3·6 % of the larvae and pupae of *Sphaerophoria scripta* were attacked. An account of the biology of *Bassus laetatorius* is given by Kelly (1914).

(2) *Homocidus*

Four species of this genus have been bred from Syrphid pupae, and of these *Homocidus signatus* Gr. was by far the most common, there being 22 (14 ♀♀ and 8 ♂♂) of this species out of a total of 36.

(*a*) *Homocidus signatus* (Grav.).

The records show that the biology of this species resembles that of *Bassus laetatorius* described above, except that ♂♂ are produced. There are at least two generations per annum; the members of the first emerge in spring and early summer, and those of the other appear during September and October. As mentioned above, little collecting was done in July and August, except in 1935, and the records for that year indicate the emergence of another generation during the latter month. It is certain that *Homocidus signatus* attacks the host before the pupal stage, for parasites emerged from puparia which had been formed in captivity.

An interesting feature is the marked preference shown by this species for selecting *Sphaerophoria scripta* and *Sph. flavicauda* as hosts. I have no records of *Homocidus signatus* parasitizing *Syrphus balteatus* or *Platychirus* sp., though other species of the genus have been bred from these Syrphidae. The following table illustrates this observation:

	No. of Syrphid larvae or pupae collected	No. parasitized by *Homocidus signatus*
Sphaerophoria scripta	350	22
Sph. flavicauda	18	3
Syrphus balteatus	399	0
Platychirus spp.	29	0

The percentage parasitism of *Sphaerophoria scripta* by *Homocidus signatus* was 6·3. The number of *Sphaerophoria flavicauda* collected was not sufficient to determine the percentage parasitism.

(*b*) *Homocidus tarsatorius* (Panz.).

The emergence of two adults of this species from puparia of *Syrphus balteatus* was recorded. One emerged at the end of August 1933, from a puparium which had been collected as such early in the same month, while the other emerged in April 1936, the host having been collected as a larva in September 1935. These records again show the occurrence of at least two generations per annum, characteristic of these Ichneumonid parasites.

(*c*) *Homocidus pictus* (Grav.).

One ♀ was bred from the puparium of *Platychirus* sp. in June 1934. The host was collected at the larval stage in October 1933.

All these observations show that the biology of the various species of *Homocidus* is very similar to that of *Bassus* spp. This is not surprising among members of the same group which attack the same hosts. Thus there are at least two generations of the parasite during the year, and the host is attacked before the pupal stage.

It is well known that *Bassus laetatorius* lays its eggs in the eggs and young larvae of the host. Dr O. W. Richards observed the ♀ of *Homocidus cinctus* (Grav.) laying in the egg of a Syrphid.

(3) *Promethus*

Two species have been bred from Syrphid puparia, namely: *Promethus monticola* Sey. and *P. sulcator* (Grav.).

(*a*) *Promethus monticola* Sey.

Nine individuals of this species were bred, and the biology is very similar to that of the other Bassines. Again the records show the occurrence of at least two generations per annum. Like the species of *Bassus* and *Homocidus* this parasite attacks the host before the pupal stage.

All the *Promethus monticola* obtained were bred from puparia of *Sphaerophoria*, eight from *Syrphus scripta* and one from *S. flavicauda*.

(*b*) *Promethus sulcator* (Grav.).

Dr O. W. Richards bred one specimen of this parasite from the puparium of *Melanostoma mellinum*. This was collected in the larval stage, on dock at Slough, Bucks.

In his description of this species, Morley (1911, p. 118) says, "Holmgren once bred it from an undetermined species of *Syrphus*, in Sweden", which is the only hint we have respecting its economy.

(4) *Gelis* sp.

One ♀ of a species of *Gelis* was bred from the puparium of *Melanostoma mellinum*. This puparium was collected on 18 August 1932 on *Alliaria officinalis* Andrz., under willow, and on 19 August a long narrow parasitic egg was noticed through the puparial skin, near the head of the pupa. On 20 August the larva of the parasite was visible through the skin, and

the adult emerged on 8 September. Thus, the only detail available regarding the biology of this parasite is that the pre-adult period lasts for about 17 days.

MOUTH PARTS OF THE MATURE LARVA OF SOME ICHNEUMONID PARASITES

(Pl. XXX, figs. 1–4)

The head of the last larval stage of some of the Bassinae was examined. In order to do this, the puparia from which the parasites had emerged were soaked overnight in a solution of caustic soda, then dissected, and the cast larval skins they contained were mounted in Faure's fluid, without staining. This method was used by Thorpe (1930, p. 404), and the nomenclature used here is, as far as possible, that given in his paper.

It was found that the mouth-parts of all the species examined showed a similar general plan, but differed considerably in detail, so that it was possible to separate and identify the genera, and also some of the species.

Bassus laetatorius (Fab.) (Pl. XXX, fig. 1).

The labial ring is well developed, broadly rounded, and produced to a sharp point at the closed end, while the tips of the "arms" on the open side are rounded. The labrum is present in the form of a sclerotized ridge, bearing sensillae, and lies between and slightly dorsal to the tips of the labial "arms". The mandibles are well defined, the apices being heavily sclerotized, but the basal articulatory part is not fully formed. A pair of structures situated laterally to the mandibles and the labial ring, are the mandibular struts. These are similar to the mandibular struts described by Thorpe as characteristic of the Ophionines (1930, p. 405), for they are "typically Y-shaped, with a long stem and short arms". "The upper arm is merely a condyle which articulates with the mandible, the lower arm...is the maxillary strut." There is no indication in this species of the labral and labial struts described by Thorpe.

Characteristic features of the larval mouth parts of the Ichneumonid parasites examined are the prominent sensillae. In this species, there are three pairs, viz., the labial sensillae situated within the labial ring, the maxillary sensillae found laterally to the arms of this labial ring, and a small pair, probably the labral sensillae, which lie near the tips of the arms and lateral to the labral ridge.

Homocidus spp.

The larval mouth-parts of four species of this genus were examined, and while those of *Homocidus tarsatorius* (Panz.) show specific characteristics, the others are indistinguishable from one another.

The presence of a pair of irregular sclerotized areas, rather like sensillae in formation, and lying latero-dorsally to the labrum, appears to be characteristic of this genus.

(*a*) *Homocidus signatus* (Grav.), (Pl. XXX, fig. 2). *H. nigritarsis* (Grav.) and *H. pictus* (Grav.) The labial ring, mandibles, labral ridge, labial sensillae, labral sensillae and maxillary sensillae, are all present. The labial ring is of the usual form, but instead of being rounded, it is rather angular, with two well-defined latero-ventral "corners", while the tips of the "arms" are narrow and almost pointed.

Apart from the slight indication of the sclerotization of the hypopharyngeal region, no other structures are seen in this species at this stage, the labial, maxillary and mandibular struts being absent.

(*b*) *Homocidus tarsatorius* (Panz.) (Pl. XXX, fig. 3). The mouth-parts of the last larval stage of this species are essentially the same as those of the other species of *Homocidus* described above, except that the tentorial structures are well developed and characteristic.

The labial ring, sensillae and labral ridge are very similar to those found in other species of the genus, but the tips of the arms are not attenuated to any extent. The "mandibular-maxillary" struts are almost circular, with the large, conspicuous mandibles articulated to the dorsal parts. Both labral and labial struts are developed, but they are small, and their shape is ill-defined.

Promethus monticola Sey. (Pl. XXX, fig. 4).

The arrangement of the larval mouth-parts of this species conforms to the general plan, and the essential features are easily noted. Labial ring, labral ridge, mandibles, labial and maxillary sensillae are present, but do not show any specific characteristics. The pair of sensillae designated "labral" in the descriptions given above are absent.

The special features of the mouth-parts of the last larval stage of this species are the well-developed tentorial structures. The labial, mandibular, maxillary and labral struts have fused to form a pair of structures which lie one on each side of the labial ring, and in which the component parts are distinct.

Chalcidid parasites

(1) *Syrphophagus aeruginosus* (Dalm.).

On 27 June 1935, a Syrphid larva, found later to be that of *Syrphus torvus* O.-S., was collected on *Pinus sylvestris* on Bagshot Heath, Surrey. The larva pupated on 2 July, and on 25 July, 2 ♂♂ and 5 ♀♀ of a parasite emerged. These have been identified as *Syrphophagus aeruginosus*, usually known as *Microterys aeruginosus* (Dalm.).

One ♀ died almost immediately, and of the others, two pairs and two separate ♀♀ were put in tubes containing larvae of *Syrphus luniger* Mg., *Sphaerophoria scripta* (L.) and *Melanostoma mellinum* (L.) respectively. 2 ♀♀ and 2 ♂♂ were dead on 29 July and the others died on 2 August. The Syrphid larvae used in this experiment pupated later, but no parasites emerged.

This parasite must attack the host before the pupal stage, since the parasites emerged from a puparium which had formed in captivity.

Dr A. B. Gahan, of the U.S. National Museum, confirmed the genus as *Syrphophagus*, and suggested that European Hymenopterists had probably described it under *Microterys*. It agrees perfectly with Mayr's description (1876, p. 723) of *Encyrtus aeruginosus* Dalm.

(2) *Pachyneuron formosum* Walker.

This Chalcidid has been bred frequently at Slough from pupae of *Sphaerophoris scripta* and *Syrphus balteatus*. The records show that there are at least two generations per annum, and it is possible that one or more occur during the summer. The adults of the first generation emerge in spring and early summer (March–June), having hibernated as ecto-parasitic larvae on the body of the pupa of the Syrphid host. The last generation emerges in September and early October. Breeding experiments conducted in the laboratory suggest that one or more generations occur between these two, and the apparent absence of corresponding generations in nature is probably due to the small amount of collecting done during July and August.

When a brood of *Pachyneuron formosum* emerged they were put in a 3×1 in. specimen tube which contained a Syrphid pupa, and also a cut and moistened raisin, fixed on a pin which was suspended from the muslin covering the open end. Several of the attempts to breed the parasite were unsuccessful, for the host was sometimes too far advanced in development to be affected by the parasite, or else the pupa was already dried up and dead. A number of the experiments were successful, and the following is an account of some of these.

On 29 May 1935, a brood of 6 ♀♀ and 1 ♂ *P. formosum* emerged, and were put in a tube containing a raisin and the pupa of *Syrphus luniger*. Females were seen to pierce the puparium with the ovipositor, but a ♂ *S. luniger* emerged on 16 June. 2 ♀♀ of this brood of parasites died on 30 May, and on 31 May the remainder were put in another tube with another pupa of *Syrphus luniger*. No parasites emerged, and when the puparium was cut open on 26 June, the pupa was found to be well advanced in development but dried up. On 3 June, these same 4 ♀♀ and 1 ♂ *Pachyneuron formosum* were still alive, and were now put in a tube with a pupa of *Syrphus ribesii*. The ♀♀ were not actually seen to pierce the puparium, and all were dead by 26 June. Two days later, 28 June, a brood of 9 ♀♀ and 4 ♂♂ of *Pachyneuron formosum* emerged from this puparium. On the same day these were sorted and put in tubes containing puparia of *Syrphus ribesii*, *S. luniger*, and *S. corollae*, which had developed from eggs laid in the laboratory. There were 2 ♀♀ and 1 ♂ *Pachyneuron* in three of the tubes, and 3 ♀♀ and 1 ♂ in the other. The ♀♀ pierced the puparia almost immediately, and all the parasites of this brood were dead by 12 July. On 22 July, 10 ♀♀ and 3 ♂♂ *P. formosum* emerged from the puparium of *Syrphus corollae*, and 2 ♀♀ from one of

S. luniger. 4 ♀♀ and 2 ♂♂ emerged from the other *S. luniger* on 25 July, while the pupa of *S. ribesii* was found to be dead when the puparium was opened on 26 July.

These parasites were now paired, and put in tubes with pupae of *S. balteatus*, *Sphaerophoria scripta* and *Syrphus luniger*. The ♀♀ were seen to pierce the puparia, and when a puparium of *S. luniger* was opened on 29 July, ectoparasitic larvae of the Chalcid were seen. The other puparia were opened too, but the Syrphid pupa had not withdrawn from the pupal skin, and so no eggs or larvae were seen, and the pupa was killed during the experiment.

On 3 June 1935, a brood of 4 ♀♀ and 2 ♂♂ of *Pachyneuron formosum* emerged from the puparium of *Syrphus balteatus* which had been collected in August 1934. These were put in a tube containing a pupa of *S. luniger*. All the parasites were dead on 28 June, but on 1 July a brood of 5 ♀♀ and 3 ♂♂ of the parasite emerged, and put in tubes containing pupae of *S. balteatus*, *Sphaerophoria scripta*, and *Syrphus corollae*. 4 ♀♀ and 1 ♂ *Pachyneuron* emerged from the puparium of *Sphaerophoria scripta* on 26 July, while the others were found to be dead.

These experiments show that the length of the pre-adult period in the laboratory is from 24 to 27 days. Laboratory conditions in summer do not differ greatly from those in the field, and so one may assume that this approximates to the length of the developmental period in nature.

The puparia of the host are attacked soon after the parasites' emergence, and the females were observed to keep the ovipositor inserted in the puparium for as long as 3 hr. The number of females in a brood always exceeded the number of males, characteristic proportions being 10 ♀♀ : 2 ♂♂, 8 ♀♀ : 3 ♂♂, 9 ♀♀ : 1 ♂, and there is one record of 20 ♀♀ : 5 ♂♂.

Earlier in the present paper it has been noted that *Sphaerophoria scripta* and *Syrphus balteatus* usually pass the winter in the larval stage, but it was also stated that *Pachyneuron formosum* hibernates as an ectoparasitic larva on the pupa of its hosts, which were the Syrphids named above. This appears to be contradictory, but the parasitized puparia were collected in August, September and early October of the previous year; if not parasitized the fly would almost certainly have emerged before the winter.

The following table shows the number of pupae collected during the months when they were to be found on cabbages, and also shows the figures on which the percentage parasitism of the puparia of *Sphaerophoria scripta* and *Syrphus balteatus* is based.

No. collected in	*Syrphus balteatus*	*Sphaerophoria scripta*
May	0	0
June	27	9
July	40	9
August	60	52
September	142	174
October	79	57
November	0	0
Total	348	301
No. parasitized	28	44
∴ % total parasitism	8	14·6
No. parasitized by *Pachyneuron*	12	12
∴ % parasitism by *Pachyneuron*	3·6	4·4

Pachyneuron formosum has been bred from the puparia of *Syrphids* by many workers. The majority of parasitized pupae recorded are those of *Syrphus balteatus*, *S. ribesii*, and (in this paper) *Sphaerophoria scripta*, but Voukassovitch (1925, p. 171) records *Syrphus vitripennis*, *S. pyrastri* (*Catabomba pyrastri*) and *Melanostoma mellinum* as hosts. I have also been able to induce the parasite to attack the pupae of *Syrphus luniger*, *S. ribesii* and *Melanostoma mellinum*, but of the Syrphids collected in nature only *Sphaerophoria scripta* and *Syrphus balteatus* were attacked. The parasite may have a preference for the puparia of these two Syrphids as hosts, but the available data is not sufficient to establish such a suggestion.

Faure (1923, pp. 255–8) has described the oviposition and development of *Pachyneuron* sp. parasitic on the pupa of *Syrphus balteatus*. Voukassovitch (1925, p. 171) records a species of *Pachyneuron* as a parasite of the Ichneumonid parasite of a Syrphid, and says that *Pachyneuron* may be a primary parasite of the Syrphid pupa, but very rarely. From the records of Dr O. W. Richards, and from my own observations, it would appear that the reverse is true at Slough, *Pachyneuron* being generally a primary parasite of the Syrphid, and occasionally a hyperparasite. Salt (1936, p. 10) agrees with this view, and Voukassovitch was perhaps dealing with another species of *Pachyneuron*. *P. formosum* is the species of this genus recorded generally as bred from Syrphid pupae.

Proctotrupid parasites (*Calliceratidae*)

(1) *Lygocerus* sp.

A brood of nine *Lygocerus* sp. emerged on 1 July from a pupa of *Sphaerophoria scripta*, the larva of which had been collected on 10 May. On examination, all these parasites were found to be ♂♂, and so no further parasitism was possible. Of these, four were dead on 13 July, and

the remainder by 17 July. The parasites were always kept in a 3×1 in. specimen tube which contained a moistened raisin for food, and had muslin over the open end. This method proved to be very successful.

Mr G. E. T. Nixon of the British Museum (Natural History) kindly identified the genus, but no specific name could be given, owing to the confusion into which the taxonomy of the group has fallen.

(2) *Trichosteresis försteri* Kieff.

Dr O. W. Richards's records show the emergence of two broods of this parasite from Syrphid puparia. The record of the first brood was published (Richards, 1930, pp. 277–8) and the host was described as "an unidentified Syrphid pupa". This is now found to be the puparium of *Sphaerophoria scripta*.

The other brood (2 ♀♀ and 1 ♂) emerged from the puparium of *Syrphus luniger* on 1 August 1933. The larva of this Syrphid was collected on cabbage on 23 June, and pupated on 26 June, so that the parasite must have attacked the host before the pupal stage.

(3) *Conostigmus* sp.

Four puparia of *Syrphus balteatus* were collected in August 1933, three on 22 August and one on 24 August. All contained larvae of the Proctotrupid parasite *Conostigmus* sp. Although kept indoors, these parasites hibernated within the Syrphid puparium and did not emerge until the summer of the next year—end of May until the beginning of July. This suggests that there is only one generation per annum of this parasite. All the parasites in one puparium died before emergence; of the others 9 ♀♀ and 1 ♂ emerged from one, and one from each of the other two.

Since the hosts were collected as puparia it is impossible to tell at what stage this parasite attacks the Syrphid.

Mr G. E. J. Nixon of the British Museum (Natural History) kindly identified the genus.

My sincere thanks are due to Prof. J. W. Munro, D.Sc., for facilities for carrying out this work at the Imperial College Field Station, Slough, Bucks, and particularly to Dr O. W. Richards for much kind help and many suggestions while the work was in progress.

I also wish to thank Mr G. E. J. Nixon and Mr J. F. Perkins of the British Museum (Natural History) for identifying the Proctotrupids and the Ichneumonids respectively, and Dr A. B. Gahan of the U.S. National

Museum for identifying and confirming the genus *Syrphophagus*. I am also indebted to the authorities of the British Museum, particularly Dr J. Smart, for allowing me to examine some Syrphid puparia in the Museum collection.

REFERENCES

FAURE, J. C. (1923). Mode de succion de *Pachyneuron* sp. Chalcidien parasite des pupes de *Syrphus balteatus*. *Rev. Path. vég.* **10**, 255.

KELLY, E. O. G. (1914). Notes on the biology of *Diplazon* (*Bassus*) *laetatorius*. *J. econ. Ent.* **7**, 294.

MAYR, G. (1876). Die europäischen Encyrtiden. *Verh. zool.-bot. Ges. Wien.* **25**, 675.

METCALF, C. L. (1911). Life histories of Syrphidae. 2. *Ohio Nat.* **11**, 397.

—— (1912). Life histories of Syrphidae. 3. *Ohio Nat.* **12**, 477, 533.

—— (1913). Life histories of Syrphidae. 5. *Ohio Nat.* **13**, 81.

—— (1916). Syrphidae of Maine. *Bull. Me agric. Exp. Sta.* no. 253, p. 193.

—— (1917). Syrphidae of Maine (2nd report). *Bull. Me agric. Exp. Sta.* no. 263, p. 153.

MORLEY, C. (1911). *Ichneumonologia britannica*, **4**. London.

RICHARDS, O. W. (1930). A proctotrupid bred from a Syrphid pupa. *Ent. mon. Mag.* **76**, 277.

SALT, G. (1936). Miscellaneous records of parasitism. *Ent. mon. Mag.* **72**, 9.

THORPE, W. H. (1930). Observations on the parasites of the pine shoot moth, *Rhyacionia buoliana* Schiff. *Bull. ent. Res.* **21**, 387.

VOUKASSOVITCH, P. (1925). Observations biologiques sur quelques insectes prédateurs des Pucerons et leurs parasites et hyperparasites. *Bull. Soc. ent. Fr.* p. 170.

EXPLANATION OF PLATES XXV–XXX

PLATE XXV

Figs. 1–8. *Syrphus luniger* Mg. Fig. 1. Side view of puparium. Fig. 2. Dorsal view of puparium. Fig. 3. Antero-dorsal view of posterior spiracular trunk. Fig. 4. Postero-ventral view of posterior spiracular trunk. Fig. 5. Lateral view of posterior spiracular trunk. Fig. 6. Upper surface of chorion, H.P. Fig. 7. Upper surface of chorion, L.P. Fig. 8. Lower surface of chorion, H.P.

Figs. 9, 10. *Syrphus ribesii* (L.). Fig. 9. Dorsal view of chorion, H.P. Fig. 10. Lateral view of dorsal edge of chorion, H.P.

PLATE XXVI

Fig. 1. *Syrphus ribesii* (L.). Side view of puparium.

Fig. 2. *Syrphus torvus* O.-S. Side view of puparium.

Figs. 3–7. *Syrphus auricollis* Mg. Fig. 3. Side view of puparium. Fig. 4. Dorsal view of puparium. Fig. 5. Posterior view of posterior spiracular trunk. Fig. 6. Lateral view of posterior spiracular trunk. Fig. 7. Dorsal view of chorion, H.P.

Figs. 8–10. *Syrphus cinctellus* (Zett.). Fig. 8. Lateral view of puparium. Fig. 9. Posterior view of posterior spiracular trunk. Fig. 10. Lateral view of posterior spiracular trunk.

PLATE XXVII

Fig. 1. *Syrphus cinctellus* (Zett.). Antero-dorsal view of posterior spiracular trunk.
Figs. 2–6. *Syrphus albostriatus* (Fln.). Fig. 2. Dorsal view of puparium. Fig. 3. Lateral view of puparium. Fig. 4. Antero-dorsal view of posterior spiracular trunk. Fig. 5. Dorsal view of posterior spiracular trunk. Fig. 6. Lateral view of posterior spiracular trunk.
Figs. 7–10. *Syrphus balteatus* (Deg.). Fig. 7. Dorsal view of puparium. Fig. 8. Lateral view of puparium. Fig. 9. Lateral view of posterior spiracular trunk. Fig. 10. Postero-dorsal view of posterior spiracular trunk.

PLATE XXVIII

Figs. 1–4. *Catabomba selenitica* (Mg.). Fig. 1. Lateral view of puparium. Fig. 2. Posterior view of posterior spiracular trunk. Fig. 3. Postero-ventral view of posterior spiracular trunk. Fig. 4. Lateral view of posterior spiracular trunk.
Figs. 5–8. *Catabomba pyrastri* (L.). Fig. 5. Lateral view of puparium. Fig. 6. Antero-dorsal view of posterior spiracular trunk. Fig. 7. Lateral view of posterior spiracular trunk. Fig. 8. Posterior view of posterior spiracular trunk.
Figs. 9–11. *Baccha elongata* (Fab.). Fig. 9. Lateral view of puparium. Fig. 10. Dorsal view of posterior spiracular trunk. Fig. 11. Dorsal view of puparium.

PLATE XXIX

Figs. 1, 2. *Platychirus scutatus* (Mg.). Fig. 1. Side view of puparium. Fig. 2. Dorsal view of posterior spiracular trunk.
Fig. 3. *Platychirus albimanus* (Fab.). Upper surface of chorion, H.P.
Figs. 4–7. *Sphaerophoria scripta* (L.). Fig. 4. Lateral view of puparium. Fig. 5. Dorsal view of puparium. Fig. 6. Lateral view of posterior spiracular trunk. Fig. 7. Postero-dorsal view of posterior spiracular trunk.
Fig. 8. *Sphaerophoria flavicauda* Zett. Lateral view of puparium.

PLATE XXX

Fig. 1. *Bassus laetatorius* (Fab.). Mouth-parts of mature larva.
Fig. 2. *Homocidus signatus* (Grav.). Mouth-parts of mature larva.
Fig. 3. *Homocidus tarsatorius* (Pz.). Mouth-parts of mature larva.
Fig. 4. *Promethus monticola* Sey. Mouth-parts of mature larva.

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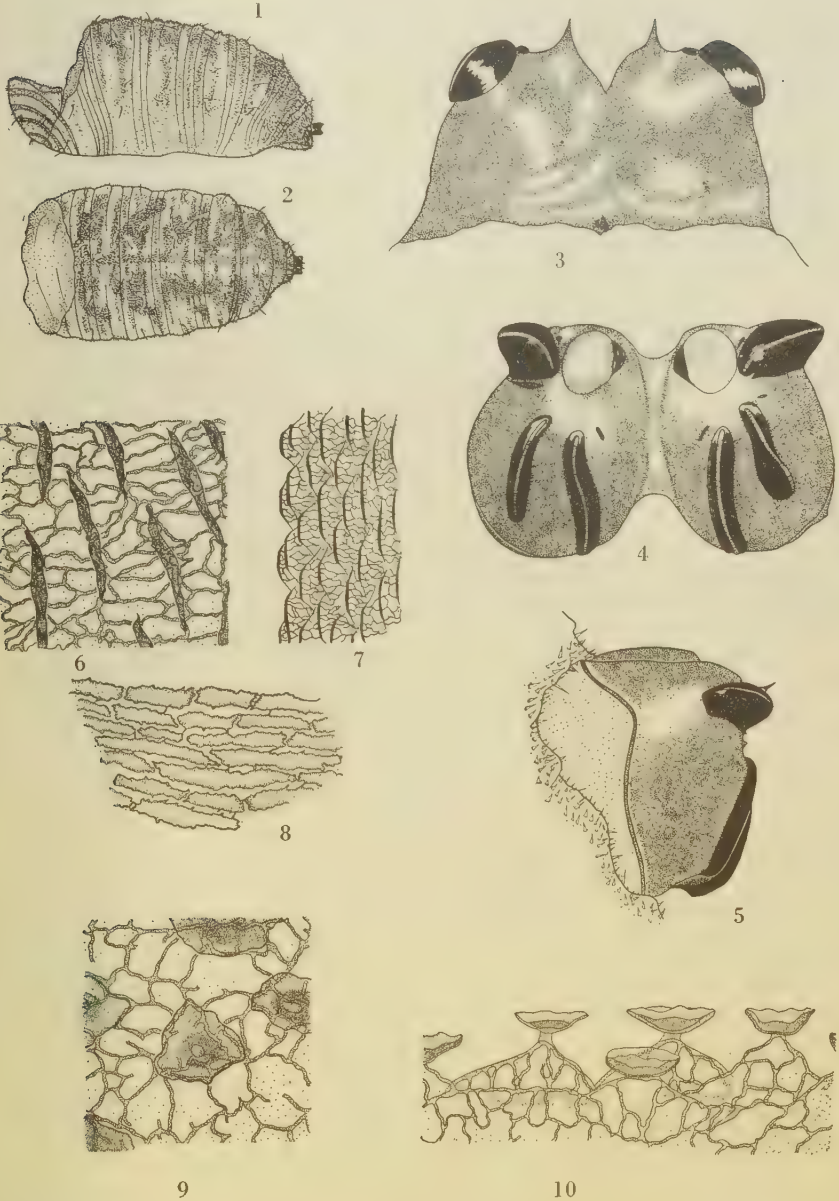

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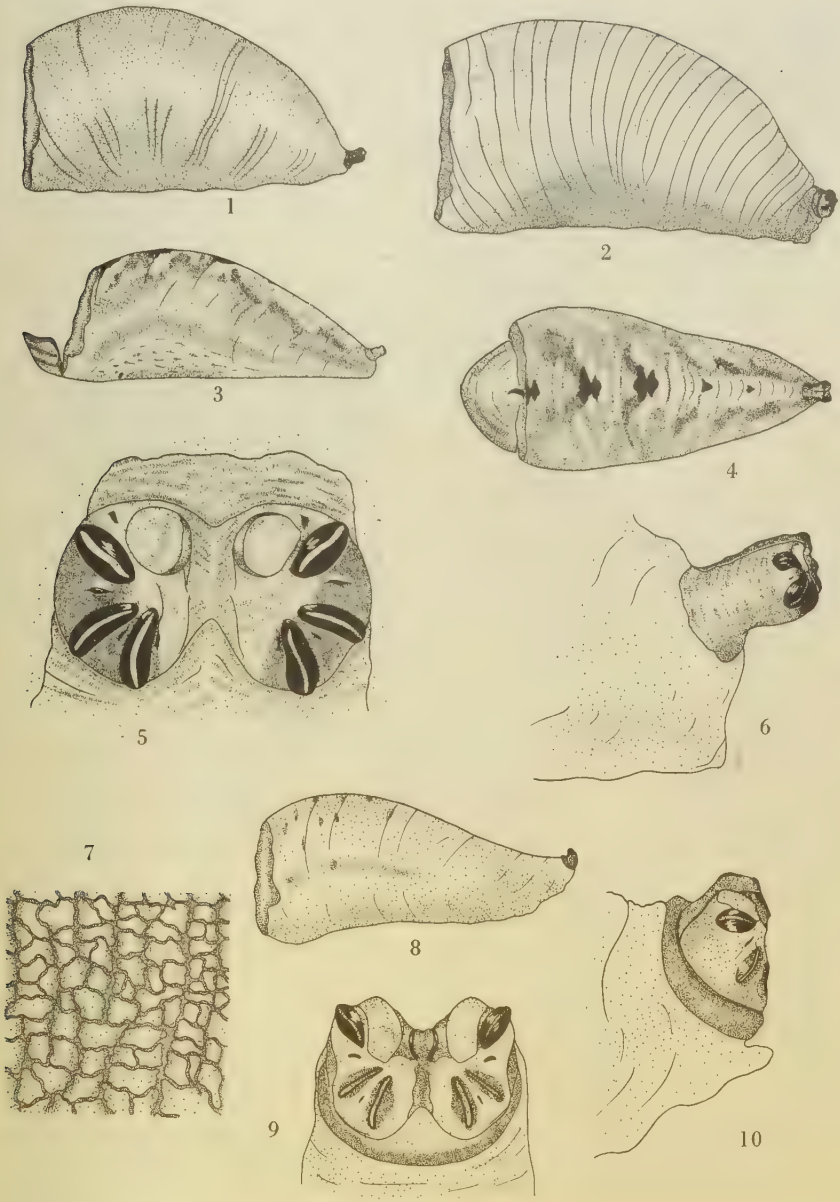

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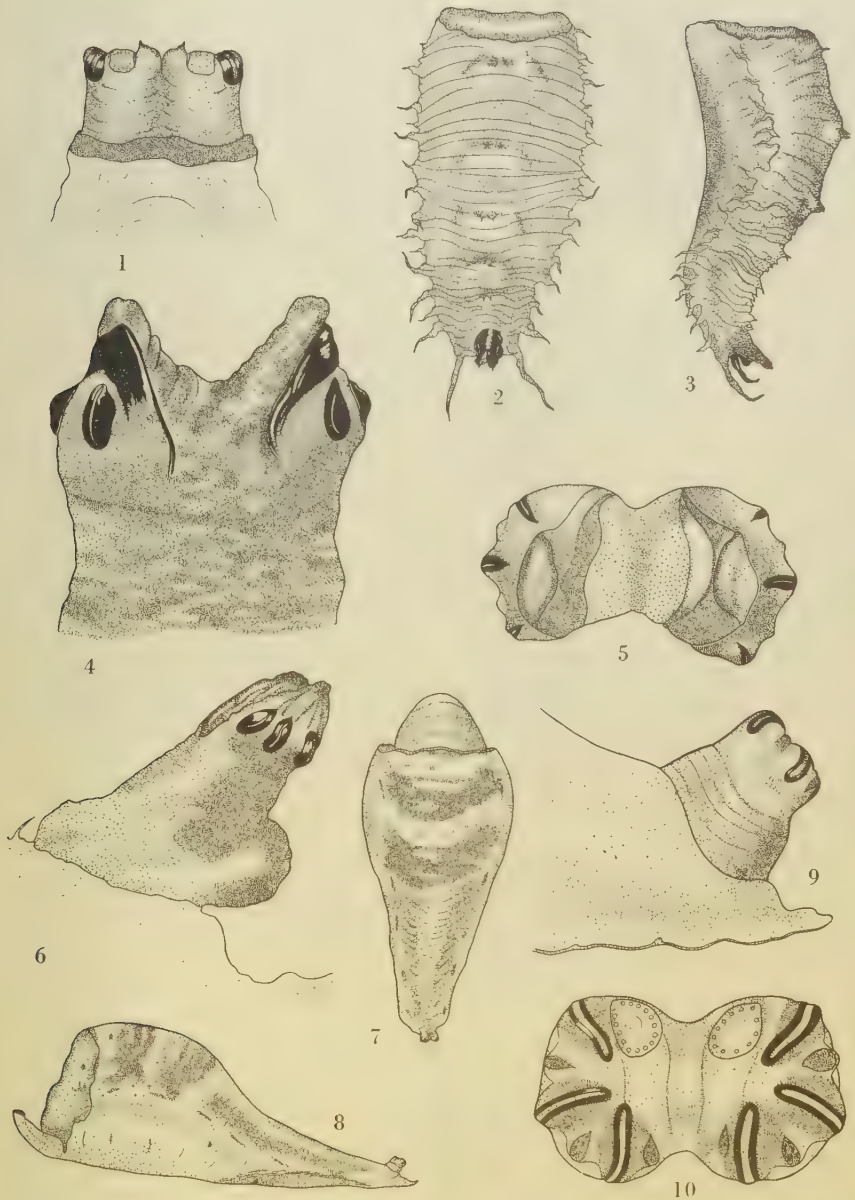

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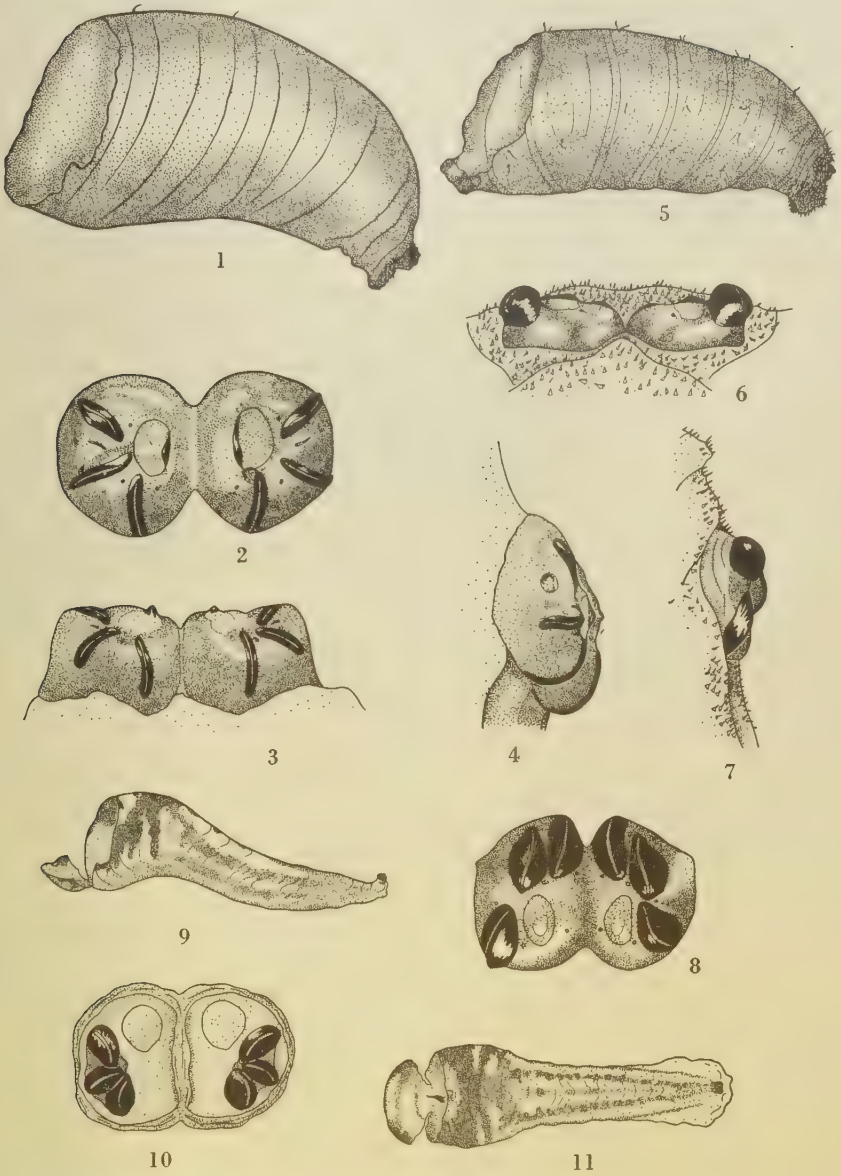

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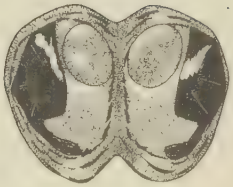

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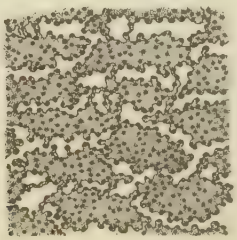

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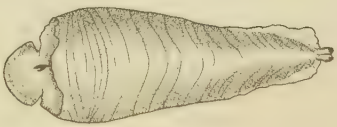

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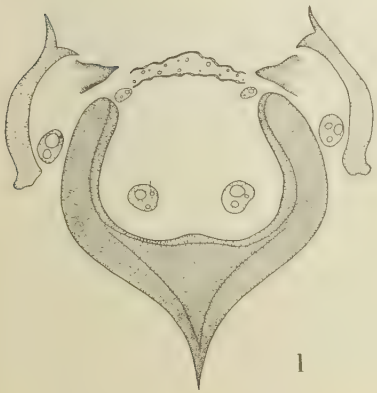

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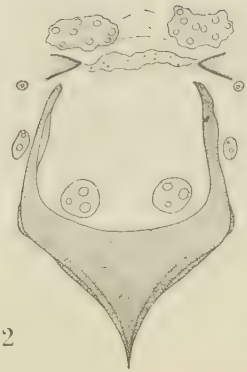

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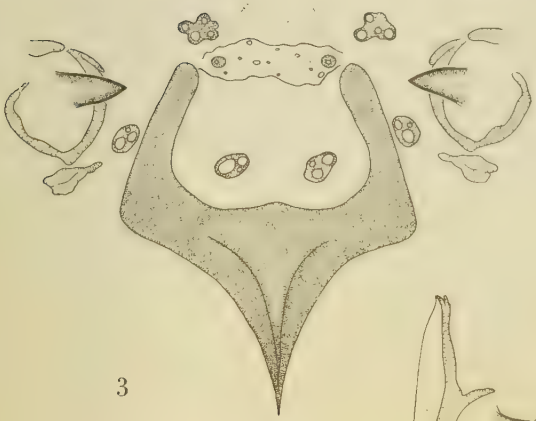

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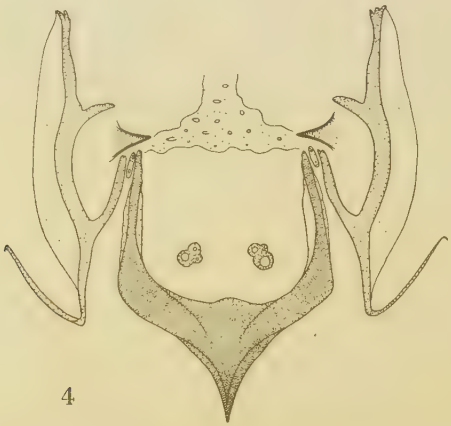

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EUNICE I. SCOTT.—An Account of the Developmental Stages of some Aphidophagous Syrphidae (Dipt.) and their Parasites (Hymenopt.) (pp. 509–532)

FACTORS AFFECTING THE RESISTANCE OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM* DUV., TO HYDROGEN CYANIDE[1]

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(With Plates XXXI and XXXII and 10 Text-figures)

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1. Introduction

Much work has been carried out in recent years on the effect of various environmental factors on the resistance of insects to fumigants. Some of the main lines of this work have been summarized in papers by Potter (1937) and Shepard *et al.* (1937). The variation in resistance of an insect

[1] Thesis approved for the degree of Doctor of Philosophy in the University of London.

with variation in its physiological state has also been studied. Knight (1925) stated that insects become more resistant to hydrogen cyanide when they become dormant or inactive, however this condition is brought about. A number of workers have shown that the resistance of an insect is different at different stages in its life history and their results are discussed in § 4 of this paper. It was proposed to investigate in detail this difference in resistance at different stages, particularly considering the application to it of Cotton's theory of the relation of resistance to fumigants and respiratory metabolism (Cotton, 1932). During the course of this work interesting problems arose which were followed up as far as time permitted.

The flour beetle, *Tribolium confusum* Duv., was chosen as a test insect. It has a short life cycle, can easily be reared under controlled conditions of temperature and humidity, and the various stages can be sieved out of the flour on which it feeds. Hydrogen cyanide was found to be a convenient fumigant, as it could be obtained very nearly pure, and a method had been established for its determination in low concentrations (Lubatti, 1935). Also, its physiological action on cellular respiration by inhibiting oxidation of the respiratory enzymes is well known from the work of Warburg, Keilin and others (Meldrum, 1933).

2. Description of apparatus used

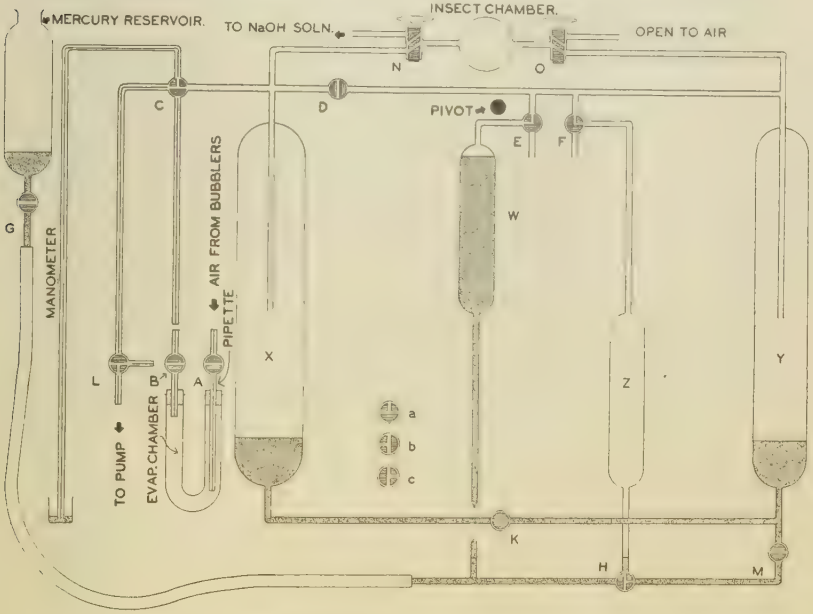
(i) *The first apparatus*

The apparatus with which most of the work was carried out was designed by Dr H. H. S. Bovingdon and is a modification of that described by him (Bovingdon, 1934). It is housed in a constant-temperature cabinet (Pls. XXXI and XXXII). This cabinet is made up of a framework of $2 \times 1\frac{1}{2}$ in. battens covered internally and externally with match-boarding, the intervening space being filled with slag-wool. The upper part of the cabinet is removable and has two double-glass windows in the top, and two felt-covered apertures on each side. The stopcocks can be manipulated through these apertures. The door in the lower part of the cabinet permits access to the tubes to which the sampling flask and the evaporating chamber are attached. The bubblers for controlling the humidity of the air in the apparatus are on the floor of the cabinet.

A diagram of the apparatus is shown in Text-fig. 1. This system and an identical one as a control are supported on a wooden framework. This is suspended about a metal rod (pivot in diagram) which is fixed to the cabinet so that by means of a crank worked by an electric motor the whole apparatus can be rocked slowly from side to side. The motor is geared down so that the framework is rocked backwards and forwards through an arc of about 15° about twice a minute. The mercury reservoir and the manometer are outside the cabinet and do not move with the rest of the apparatus.

X and *Y* are two large glass flasks of approximately 3 and $1\frac{1}{2}$ l. capacity respectively. These form the main gas reservoir and flask *X*, in addition, acts as a mixing

chamber. They are connected by a tube at their lower ends and contain about 150 c.c. mercury. At the top they are connected by a straight tube and also through the insect chamber. The latter is a hollow glass sphere, about 100 c.c. capacity, closed by a ground-glass stopper (not shown in the diagram), and with two side tubes each supplied with a two-way stopcock. As the apparatus is rocked from side to side the mercury rises and falls alternately in the two flasks *X* and *Y* and pumps the gas backwards and forwards through the insect chamber or the bypass, so that there is a constant circulation of gas. To avoid one pocket of gas going backwards and forwards, the tube from the insect chamber is continued half-way down the large flasks.



Text-fig. 1. Diagram of first apparatus. (See text for explanation.)

By allowing mercury to flow from the outside reservoir (which can be raised and lowered) into flask *W*, and from *W* to *Z*, the pressure in the apparatus can be restored to atmospheric after removing a sample of the gas for analysis, and by turning the appropriate stopcocks the insect chamber can be flushed out with the fumigant.

All the flasks and connecting tubes and stopcocks are of glass, and, where joins are necessary, butt connexions are made and joined with rubber pressure tubing. With the exception of about 3 in. of rubber tubing between the apparatus and the manometer no rubber is actually exposed to the gas. The apparatus is vacuum-tight and a Gaede rotary oil pump is used to evacuate it.

The hydrogen cyanide is measured in small graduated pipettes exactly as described by Bovingdon. The pipette is so calibrated that quantities of liquid hydrogen

cyanide corresponding to a range of concentrations of from 0·06 to 2·0 mg./l. can be measured out roughly. This process is referred to throughout this paper as "dosing". The accurate measurement of the gas concentration is described later.

The apparatus is heated by electric resistance heaters controlled by a bimetallic thermostat.

The temperature of the insect chamber did not vary more than $\pm 0{\cdot}2^{\circ}$ C. during an experiment. The humidity is controlled by passing the air entering the apparatus through six gas-washing bottles containing either potassium hydroxide or sulphuric acid solution. These solutions are made up to a certain specific gravity which will give the air the desired humidity. The specific gravity of these solutions is checked at intervals and if necessary they are replaced. When sulphuric acid was used as the principal means of controlling the humidity, two extra bottles of potassium hydroxide were added to absorb carbon dioxide so that these experiments might be comparable with those in which potassium hydroxide alone was used. The first four gas-washing bottles were ordinary Dreschel bottles. The next two had sintered glass bubblers, and these were followed by an empty Dreschel bottle and a tube of glass-wool to trap any spray.

The circulation of the gas was tested by attaching a bubbler—previously calibrated—to the open end of the insect chamber and observing the number of bubbles passing per minute in one direction. The rate of flow was about 7 l./hr., which corresponded to a linear velocity through the insect chamber of the order of 5 m./hr.

A sample of the gas in the apparatus was taken at the beginning and end of each test. The sampling flask (shown attached to the apparatus in Text-fig. 2) was a cylindrical glass vessel about 3 cm. in diameter and with a capacity of about 120 c.c. A right-angle stopcock, with a removable key, is fused to one end of the flask so that it could be opened or closed to the apparatus or vacuum pump, and afterwards the key could be removed and titration carried out directly into the flask.

Procedure of each test.

(1) All mercury is run out of flasks *W* and *Z*, by opening stopcock *G*, lowering the mercury reservoir and turning stopcocks *E* and *F* to open flasks *W* and *Z* to the air.

(2) Stopcocks *G*, *N*, *K* and *M* are closed; *O* is open to the air, *D* is opened, *C* is turned to position (*a*), *L* to position (*b*), *H* to position (*c*), and *E* and *F* turned so that flasks *W* and *Z* are in communication with the rest of the apparatus, which is now evacuated. Meanwhile, the hydrogen cyanide is taken up in the small pipette and the latter inserted in the evaporating chamber and cocks *A* and *B* are closed. The chamber is then placed in position, one tube being connected to the apparatus and the other to the bubblers; both connexions are made with butt joints connected with rubber pressure tubing.

(3) Stopcock *C* is turned to position (*b*) so that the tube *B–C* is evacuated; *D* is closed, *L* is turned to the position shown in the diagram, and *C* is turned to position (*c*). *B* is opened and the hydrogen cyanide, volatilized under the reduced pressure, is admitted to the flask *X*. The gas in this flask is then brought to atmospheric pressure by slowly opening stopcock *A* and admitting air of the desired humidity.

(4) Stopcock *A* is closed and *D* is opened, thus admitting the well-mixed gas in flask *X* to the rest of the apparatus. Air is again allowed to enter by opening stopcock

A until the whole apparatus contains a gas mixture at atmospheric pressure. Stopcock *B* is then closed.

(5) The insects, previously placed in their container, are suspended from a hook in the stopper of the insect chamber. Stopcock *K* is opened and the rocking motor started. An interval of about 15 min. is allowed for the mixing of the gas and for the insects to settle down whilst still in air.

(6) The rocking motor is stopped: stopcock *D* is closed and *O* and *N* turned so that there is now a passage through the insect chamber from flask *Y* to a bottle containing a strong solution of sodium hydroxide. Stopcock *G* is opened and the reservoir raised so that mercury flows from the latter to flask *W*. The air in the insect chamber is thus displaced by the fumigant. As flask *W* has approximately four times the volume of the insect chamber it may be assumed that the gas mixture in the latter is similar in composition to that in the rest of the apparatus. This operation takes about a minute. Stopcock *N* is then turned so as to put the insect chamber and flask *X* in communication, the rocking motor is started and the time is noted. An ordinary watch is used to time the tests. The stopcocks are now in the positions shown in Text-fig. 1.

(7) The gas-sampling flask, containing 5 c.c. of *N*/20 sodium hydroxide, is attached to the side tube of cock *L*, stopcock *C* is turned to position (*b*), *L* to position (*c*) and the evacuating pump switched on. When the manometer indicates a pressure of only a few millimetres of mercury, stopcock *L* is turned to position (*a*) and *C* is also turned very slowly to position (*a*), so that gas from the apparatus enters the sample flask. The latter is then closed by turning the key and the pressure of the gas noted on the manometer. Mercury is then run from flask *W* into flask *Z* by turning stopcock *E* so as to open *W* to the air and turning *H* to position (*b*). When atmospheric pressure is restored, *H* is turned back to position (*c*), *C* is turned to position (*c*) and the sampling flask removed. After the addition of 2 c.c. of a 7 % solution of sodium bicarbonate the sodium cyanide is titrated with standard iodine solution using 0·5 c.c. benzene as indicator.

(8) At the end of the test the rocking motor is stopped, stopcocks *O* and *N* are closed and the insects removed. A second gas sample is taken at the end of each test. A similar procedure to that described in paragraphs (1) and (2) is followed and the apparatus is evacuated and air readmitted.

Except that the hydrogen cyanide was omitted, the control insects were given exactly the same treatment.

The concentration of hydrogen cyanide in mg./l. is calculated from the mean of the two titrations. Usually there was a loss of about 1–5 % of hydrogen cyanide due to absorption by the insects, their container and stopper-grease. If the loss exceeded this, the test was not usually included in the working out of results. Certain tests, however, unavoidably gave a very high loss. These are referred to later (§ 7).

Accuracy of titrations of the sodium cyanide.

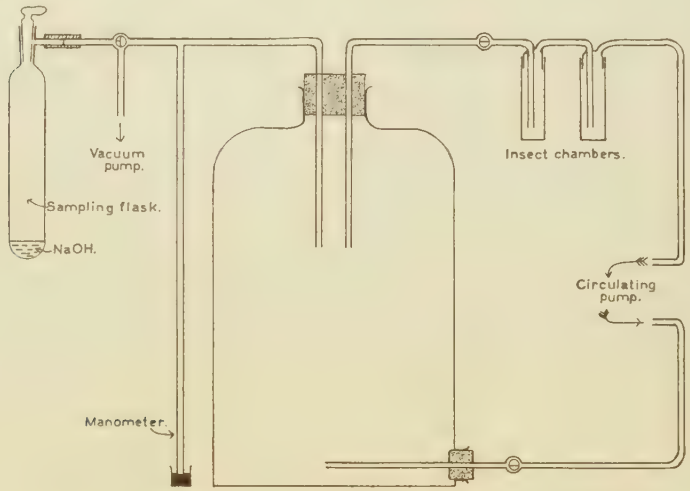
Two solutions of iodine were used, one containing about 0·5 and the other about 0·25 mg./l. The accuracy of this titration ranged from about ±6 % at the lowest concentrations to less than ±1 % at the highest.

(ii) *The second apparatus*

The apparatus described above had certain disadvantages, and for later work it was replaced by one in which some of these disadvantages were overcome. The chief of these were:

(1) Its small volume (5·5 l.) made accurate dosing difficult, and even normal losses of hydrogen cyanide formed quite a large proportion of the total amount of hydrogen cyanide present.

(2) The number of stopcocks and interconnexions made it difficult to clean and necessitated several butt joints covered with rubber tubing. The large amount of mercury was expensive, and also laborious to clean. Also it was later found to be toxic to insects (Gough, 1938*b*).



Text-fig. 2. Diagram of second apparatus. (See text for explanation.)

A diagram of the second apparatus is shown in Text-fig. 2. It was used for all the experiments on the sixth and seventh filial generations, described in § 5, and all the experiments described in § 6. It was built up in a constant temperature and humidity room, so that there was no need to control these factors further. In order to remove carbon dioxide from air entering the apparatus, the air was bubbled through a solution of potassium hydroxide of such a strength that the humidity was not altered. A 20 l. aspirator formed the main gas reservoir. This was sufficiently large to minimize the proportional losses of hydrogen cyanide caused by the presence of adult beetles (see § 7). Owing to the possibility of breakage it was not advisable to evacuate it completely, so that after each test it was half evacuated and refilled with air four times.

The top and bottom openings of the aspirator were closed with a rubber bung covered with tinfoil so as to prevent the absorption of hydrogen cyanide. Each carried a glass tube leading to the insect chambers and the mercury circulating pump. The latter, a modified Leach type of pump, contained only a small quantity of mercury,

and this could be covered with some inert substance if necessary. This was not done as it was not realized at this stage that the mercury vapour might be toxic. The insect chambers were small gas washing bottles of about 15 c.c. capacity. They could be shut off from the rest of the apparatus by stopcocks. A second tube in the top bung led to the manometer and to a threeway cock. The sampling flask was attached to one arm of this and the other arm was either connected to the evacuating pump or could be used for the attachment of the evaporating chamber.

Procedure of experiment.

The aspirator was first partially evacuated, the cocks to the circulating pump being closed. The hydrogen cyanide was then admitted, using the same method as described above, and air swept through the evaporating chamber to bring the apparatus to atmospheric pressure. The circulating mechanism was then started after the appropriate stopcocks had been opened. After about half an hour the pump was stopped, the stopcocks closed and the insects placed in the insect chamber, which already contained hydrogen cyanide. Some of this was inevitably lost whilst the insects were being introduced. The small size of the chambers and the relative efficiency of the pump, ensured that the gas concentration in the insect chambers would reach that in the rest of the apparatus within a few minutes of the circulating pump being restarted. This arrangement, of course, did not give the insects the opportunity of settling down. Gas samples were taken exactly as described for the first apparatus. Control experiments had to be carried out in the same apparatus when fumigations were not being performed: they were only done occasionally.

3. Technique

(i) *The breeding of large numbers of insects of known stage, age and sex*

The beetles were bred on a roller-ground wholemeal flour, which was sieved through no. 9 bolting silk. The flour was first sterilized and then exposed on shallow trays in the constant temperature and humidity room for about a fortnight. During this period, which allowed it to come into equilibrium with the conditions in the room, it was covered with muslin to prevent reinfestation. All the cultures were kept in this room at 27° C. and 60 % relative humidity, and all fumigation tests were carried out under these conditions.

The following technique was employed to obtain the various stages at a known age. The figures in brackets refer to the approximate duration of the stage in days.

Eggs (6). Cultures of about 200 adults, between 1 and 2 months old, were made up in glass jars, about half full of flour. The adults were removed every day by means of a coarse sieve and the flour then sieved through no. 6 bolting silk, which retained the eggs. The adults were then replaced in the flour, which was changed once a week. These cultures were never kept more than 1 month. From one of these cultures it was possible to obtain about 300 eggs per day.

Larvae (24). Eggs laid within 24 hr. of one another, were made up into cultures, so that the age of the resulting larvae was known within a day or so, since nearly all the eggs hatched on the same day. The larvae were fumigated 26 days after the eggs were laid, or 20 days after hatching. At this age the larvae are in the last instar, but they are still active and cling to paper when placed on it. A day or so later, in the prepupal period, they become inactive and do not cling to paper. It is not known for

certain whether they had ceased feeding at that age, although nearly all the controls pupated successfully. Owing to individual variation in rate of development, the larvae from one culture were not all in the same instar on the day they were to be fumigated, but it was not difficult to select only active larvae, in the last instar. It was more satisfactory to do this, using a separate culture for each test, rather than selecting from a mass culture, since no individuals, which might be used for future tests, were disturbed.

Pupae (8). About 200 adults were placed in a large glass jar, about one-third full of flour, and allowed to lay eggs. After a fortnight, the adults were removed, and the eggs and those larvae which had already hatched left to develop. When the pupae formed they were sieved out daily with a coarse sieve, and sorted from the larvae, which were replaced. The pupae were then divided into males and females, and, either fumigated immediately, or left in an open tube in the constant temperature room until they were required.

Adults (several months). When 0–1-day-old adults were required, several hundred pupae of all ages were divided into males and females and the resulting adults fumigated together, but in separate containers, on the day of emergence. Several attempts were made to mark the adults after emergence from pupae of known sex so that they could be easily separated again after mating. The only satisfactory method, of those tried, was the removal of the hind tarsi from one leg in one sex, and from the opposite leg in the other. This operation, which was carried out several days before fumigation, took a long time if several hundred beetles were required, and it was open to the usual objections to the mutilation of experimental animals. Finally, it was found very easy to sort the sexes after fumigation, by gently squeezing the end of the abdomen with a pair of forceps and examining the extruded genitalia under a binocular microscope or even with the naked eye. In dead or stupefied females the genitalia were frequently extruded after fumigation. Usually, this examination was carried out the day after fumigation, but if it had to be delayed for a few days so that the dead beetles dried up, the same process could be performed after boiling the insects in a weak solution of potassium hydroxide.

(ii) *The gross effect of hydrogen cyanide and the criterion of death in each stage*

Hamlin & Reed (1927) drew attention to the necessity of keeping fumigated insects under observation for some time after fumigation, the period depending on the insect. Many fumigated insects, which are apparently dead, recover later and others, which are apparently unaffected, may die later. This second possibility was not observed to occur in the present work.

After fumigation, the test insects and controls were transferred to tubes in the constant temperature room. Larvae and adults were supplied with flour, and a small piece of paper was put in with the eggs and pupae so that the resulting larvae or adults could crawl upon it.

Eggs. A certain percentage of the control eggs failed to hatch. This proportion varied from 0 to 36 %, with an average of 17 % in the 0–1-day-old eggs, and from 3 to 25 %, with an average of 10 % in the 3–4-day-old eggs. Because, sometimes, at low concentrations more eggs survived among those fumigated than among the controls, instead of correcting for the percentage survival of controls in each batch of eggs, it was decided to use the average percentage survival when computing the net kill in

each test. The following method, suggested by Bliss (1935*a*), was used for this purpose. The number of individuals used in a particular test is multiplied by the proportion alive in the untreated controls. This gives the net number of insects exposed to the poison. When the number surviving the treatment is subtracted from this net total, the difference is the number of killed, and the number killed, multiplied by 100, and divided by the net number exposed is the percentage killed. About 100 eggs each were counted out for the fumigation and control tests. When the young larvae hatched they were removed and counted daily. Occasionally a larva hatched but died afterwards: this was not included among the survivors. There was a delay of 1–3 days in the hatching of these survivors compared with the controls.

Larvae. About sixty larvae were used in each test, another sixty from the same culture being used as controls: there was no mortality among the latter. As the test larvae were ready to pupate, those which formed an apparently normal pupa were counted as survivors. The larvae which died were torpid for a day or so after fumigation. Subsequently dark blotches appeared in various places, usually starting on the thorax and spreading over the whole body, until a black shrivelled skin was left. No larvae, which were active immediately after fumigation, died later.

Pupae. About sixty pupae were used in each test and an equal number of controls. Frequently pupae which had been fumigated, gave rise to adults in which the elytra were undeveloped and which were only able to move their legs feebly and, because of this, only pupae which formed an apparently normal adult, capable of walking normally, were counted as survivors. No account was taken of a slight mortality of about 1–2 % in the controls. The emergence of adults from pupae which survived fumigation was delayed from 1 to 7 days. Some rough experiments were carried out on the egg production of these survivors, and it appeared that they at first produced only a few eggs and those not viable. After 2 or 3 weeks they produced viable eggs in normal numbers.

Adults. There was no mortality amongst the control adults, except for one lot which are dealt with in § 7 of this paper.

At the time of fumigation, none of the 0–1-day-old adults had darkened to the rich brown colour typical of older beetles. Beetles which had only just emerged and in which the elytra were still white were not used. After even the lowest concentrations to which the 0–1-day-old adults were exposed, all were stupefied at the end of the fumigation. The survivors gradually recovered about 3–7 days afterwards.

The older adults, 14 and 28 days old, behaved quite differently from the young ones. At the end of a test it was almost possible to sort out those which were alive and those which were dead, and only one or two of those supposed dead recovered later. Immediately after the fumigation, the survivors frequently were unable to co-ordinate their walking movements though they became normal on the following day. Whether this difference between young and older adults is due to a difference of age or to a difference in the method of experiment is discussed in the next section.

(iii) *Containers used for the insects during fumigation*

Several different types of containers were used to hold the insects during the fumigation: these included bags of muslin or bolting silk and short lengths of glass tubing with each end covered with bolting silk. Eventually, cylindrical iron-wire gauze cages, about 1 in. long and 0·5 in. in diameter, were found to be most con-

venient. They were strong, non-absorptive and had a large surface to which the insects could cling. Their mesh, 20 to the inch, was sufficiently wide not to affect diffusion and yet sufficiently close to ensure the retention of all stages except the eggs. For these, very small bags, made of no. 6 bolting silk, were used.

(iv) *Analysis of results*

Bliss's method (1935*a*, *b*) was used in the present paper to compare the resistance of different stages or groups of insects. All the tests were performed at 27° C. and 60 % relative humidity, and 1½ hr. was the standard time of exposure.

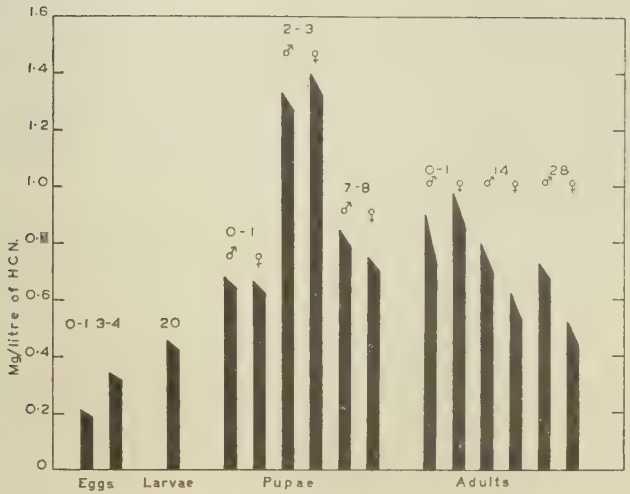
As never more than about 100 eggs were used in each test, and rarely more than sixty individuals of any other stage, it was thought unnecessary to work out the percentage mortality to more than the nearest 1 %. When converted into probits the first three significant figures were taken. Although at very high percentage kills the third figure of the probit value varied quite considerably with a 1 % difference, it was thought best to be consistent, especially as tests at high concentrations had very little weight.

Throughout this paper the word "test" has been used to describe the exposure of a certain number of insects of a given stage under given conditions to a certain concentration of fumigant. A number of such tests on the same stage, under the same conditions, has been called an experiment. It should also be noted that the word "sample" refers to a sample of the gas mixture to which the insects were exposed and not to a sample of the insect population.

4. Relative resistance of different stages to hydrogen cyanide

Table I and Text-fig. 3 summarize the results of experiments performed to determine the relative resistance of different stages of the flour beetle to hydrogen cyanide. Graphs showing the relationship of (1) percentage mortality and concentration of hydrogen cyanide and (2) mortality expressed as probits and log concentration have been plotted in Text-figs. 6–8, and regression lines for the probit/log concentrations have been calculated. The concentrations corresponding to a 50 % kill for each stage investigated have been computed, together with their limits of error, from equation (28) of Bliss's paper (1935*b*). The statistic "t" in this equation was read from Fisher's Table III (Fisher, 1932) at $P = 0{\cdot}05$. The limits of error are indicated in Text-fig. 3 by the differences in length of the boundaries of each black area.

If the value for χ^2 is smaller than the value corresponding to a probability of 0·05 in Fisher's Table III the data may be considered consistent with the straight line that has been fitted to them. If the value for χ^2 is greater than the value corresponding to a probability of 0·05 the variation about the line that has been fitted is significantly greater than would be expected from simple fluctuation in sampling, and this is denoted in the table by S.



Text-fig. 3. Concentrations of hydrogen cyanide necessary to give a 50 % kill of different stages of *Tribolium* at 27° C and 60 % relative humidity for 1½ hours. The limits of error within which the concentration for each stage has been determined are indicated by the differences in length of the boundaries of each black area. The figures above each stage refer to the age of that stage in days.

Table I. *Summary of data of experiments on different stages*

Stage		*n*	χ^2	Mg./l. of hydrogen cyanide to give 50 % kill
0–1-day-old eggs		12	48·7128 (S.)	0·195 ± 0·007
3–4-day-old eggs		13	70·7716 (S.)	0·326 ± 0·007
20-day-old larvae		15	126·4629 (S.)	0·439 ± 0·015
0–1-day-old pupae:	Male	12	103·2521 (S.)	0·659 ± 0·015
	Female	8	68·9811 (S.)	0·639 ± 0·018
2–3-day-old pupae:	Male	13	126·8112 (S.)	1·296 ± 0·023
	Female	11	64·6612 (S.)	1·363 ± 0·035
7–8-day-old pupae:	Male	17	217·5798 (S.)	0·817 ± 0·022
	Female	19	324·7490 (S.)	0·729 ± 0·020
0–1-day-old adults:	Male	6	4·6915	0·810 ± 0·085
	Female	8	8·3724	0·919 ± 0·060
14-day-old adults:	Male	5	4·8909	0·750 ± 0·031
	Female	5	32·9446 (S.)	0·569 ± 0·038
28-day-old adults:	Male	9	29·2491 (S.)	0·703 ± 0·025
	Female	8	30·7116 (S.)	0·479 ± 0·040

The limits of error above and below the mean are not necessarily quite the same. For convenience the results are expressed as ± the greater error.

Cotton (1932) states that in most holometabolic insects, the adult has the highest rate of metabolism, followed, in order, by the larva and the pupa. In support of this the only data quoted were those of Batelli and Stern on flies. Cotton demonstrated experimentally that the order of resistance to carbon disulphide of different stages of *Tribolium confusum* Duv. and *Ephestia kühniella* Zell. was pupa (most resistant), larva, adult. He deduces from this, that, other conditions being equal, a difference in respiratory rate between the various stages corresponds to a difference in susceptibility to fumigants. This suggestion has been investigated in more detail by Lindgren (1935), who studied the order of resistance of different stages of *Tribolium* to various fumigants, with the following results:

Carbon disulphide: Egg—pupa—adult—larva (low humidity).
Pupa—egg—adult—larva (high humidity).
Chloropicrin: Egg—pupa—adult—larva.

There was little difference between the resistance of the adults and larvae towards either of these fumigants.

Ethylene oxide: Pupa—adult—larva—egg.

All the above refer to tests on groups of individuals of various ages. The present writer found the following order of resistance to *hydrogen cyanide*: pupa—adult—larva—egg. This is true if the most resistant age tested of both pupae and adults be taken as representative of those stages.

If the egg be neglected, the order of resistance to all these poisons is pupa—adult—larva. According to Lindgren (1935) the following is the order of the rate of metabolism of the different stages (Highest first):

Larva—adult—pupa—egg (without flour).
Old adult—larva—young adult—pupa—egg (with flour).

In the table in which he compared respiratory rates to susceptibility to fumigants, Lindgren showed the figures of respiratory rates referring to insects with flour, though, as his fumigations were presumably carried out on insects without flour, it would seem more reasonable to use the corresponding respiratory rates. If this be done and the egg is not included, there is a correlation between respiratory rate and susceptibility to fumigants, that is, the stage with the highest metabolism is least resistant. Without further work in which fumigation tests and measurement of respiratory rates are performed under exactly the same conditions, and on insects from the same stock, it would be unwise to be

dogmatic on this point. It must be remembered, however, that (1) rate of metabolism is usually measured per gram live weight, whereas resistance is usually measured independently of size, (2) that the rate of metabolism is increased enormously by increase in activity. It may, however, probably be considered significant that in *Tribolium*, the order of resistance, pupa—adult—larva, is common to a number of fumigants. It is interesting to note that in *Tribolium*, the egg, which has the lowest rate of metabolism, is sometimes the most, and sometimes the least resistant stage. It may be possible to group fumigants according to this differential action on the egg and on the other stages. Gough (1938*a*) has pointed out that the egg of the bed-bug is more resistant to sulphur dioxide than any other stage, whereas Bovingdon (1935) finds that to hydrogen cyanide the egg is the least resistant stage of this insect.

In considering the relationship of metabolism to resistance at different times within one stage, discussion is again somewhat fruitless in the absence of reliable and apposite quantitative data. For various insect eggs, Fink (1925), Melvin (1928), Bodine (1929) and Burkholder (1934), have shown that there is an early "formative" period, which lasts a varying length of time according to the species of insect and is characterized by a low respiratory rate, and a later period, in which the metabolism increases up to the time of hatching. If this can be generally applied one would expect that the 0–1-day-old eggs of *Tribolium* would have a lower rate than eggs 3–4 days old, and according to Cotton's theory would be more resistant. Actually, as can be seen from Text-fig. 3, it was found that the older eggs are the more resistant to hydrogen cyanide. On the other hand, the resistance of the egg of the bed-bug to sulphur dioxide decreases with its age (Gough, 1938*a*). Uchida (1938) found that the susceptibility of eggs of *Chaetodacus dorsalis* Hend. to carbon disulphide increased as they developed.

The U-shaped curve mentioned by Wigglesworth (1934), which is characteristic of pupal metabolism, was obtained by Lindgren for pupae of *Tribolium*. He found that the susceptibility of pupae to chloropicrin and ethylene oxide also followed similar curves. The present author likewise found that recently formed pupae and those just about to emerge were much more susceptible than those in the middle period. Similar results were obtained by Uchida (1938) for pupae of *Chaetodacus dorsalis* Hend. and carbon disulphide.

Park (1936) found that females of *Tribolium confusum* have a higher respiratory rate than males. This may be correlated with the higher susceptibility to hydrogen cyanide of the females. Lindgren (1935)

records a considerable difference in the rate of metabolism of old adults with and without flour. As young adults did not exhibit this difference, he suggests that the adults do not feed for a few days after emergence. He found that the young adults were more resistant to carbon disulphide. The present writer found later (see § 6) that if older beetles were removed from flour about 1 hr. instead of the usual 20 min. before their fumigation they were much more resistant. Furthermore, several were stupefied at the end of the fumigation and recovered later like the 0-1-day-old adults. As the 0–1-day-old adults were never given the opportunity of feeding it was thought that this might account for their apparently different reaction. Accordingly, tests were carried out in the second apparatus, some on 0-1-day-old adults, which were supplied with flour as pupae, and others on 0-1-day-old adults which had no flour, as in the original experiment. The results were not significantly different as can be seen from the following figures expressed as mg./l. of hydrogen cyanide to give 50 % kill:

0–1-day-old adults emerged in flour	...	$0{\cdot}629 \pm 0{\cdot}026$
0–1-day-old adults without flour	...	$0{\cdot}625 \pm 0{\cdot}051$.

For several reasons these experiments are not quite comparable with the original ones, and also the males and females were not separated, as it appeared from other experiments that the two sexes did not differ greatly in resistance at this age.

The results described in § 6 suggest that under natural conditions there might not be such a great difference in resistance between young and old adults as would appear from the experiments described in this section. Further work is needed to elucidate this point.

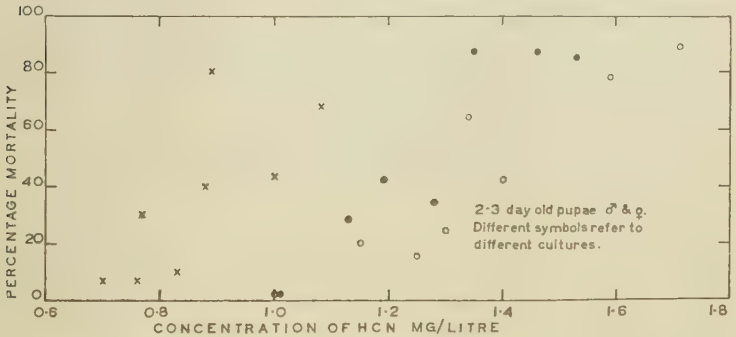
Before leaving this question of the relationship of metabolism to resistance to poisons, attention should be drawn to a tabular summary of it in a recent paper by Busvine (1938).

5. Inheritance of resistance to hydrogen cyanide

Pupae, 2–3 days old, as can be seen from Text-fig. 4, exhibited a very wide range of variation in resistance. When the data were examined a difference was found between pupae obtained from different cultures.

These tests on different cultures were carried out at different times after a sufficient interval had elapsed for the results of tests carried out on pupae from one culture to be known. While the possible reasons for this variation are discussed in § 8, it seemed worth while investigating the possibility of inheritance of different degrees of resistance. Several

American workers have suggested that such a phenomenon would account for the increase in resistance to insecticides of certain scale insects (Melander, 1914; Quayle, 1922; Woglum, 1925). Boyce (1928) reported that after seven generations of breeding from individuals of *Drosophila melanogaster* which survived a highly toxic concentration of hydrogen cyanide, there was an average of 2 % more flies surviving than among the controls, when both were exposed to the same concentration of gas. He obtained a similar result for *Aphis gossypii*. In his experiments, insects which were highly susceptible were all killed, and offspring were only obtained from those flies which survived a high concentration of fumigant.



Text-fig. 4. Percentage mortality—concentration data for 2–3 days old pupae.

The experiments here described were so arranged that offspring were obtained from known beetles whose degree of resistance was then determined.

Eighty-four pairs of adult *Tribolium confusum* of the same age were made up into separate cultures and allowed to reproduce. The cultures were made up in 3 × 1 in. glass tubes, each about half-full of flour and covered with muslin. After 17 days the adults were removed and exposed for 1½ hr. to a concentration of hydrogen cyanide which was calculated to kill about 40 % of the beetles under the given conditions. Each pair of beetles was enclosed in a small separate cage, and twelve pairs were fumigated at a time. The cages were made by rolling up rectangles of iron wire gauze into cylinders about 5 mm. in diameter and 15 mm. long. One end was closed by pinching it with a pair of pliers and the beetles were dropped in. The other end was then closed in the same way. By stringing these cages on to a length of fine iron wire they could be kept in a known order and the difficulty of marking each cage dispensed with. Two

fumigations were performed daily until all the adults had been exposed to concentrations of hydrogen cyanide ranging from 0·63 to 0·66 mg./l. After fumigation, each pair was removed from its cage, examined, and transferred to a separate tube containing flour and kept in the constant temperature room. The following day they were examined for any further survivors. Where both the male and the female had survived this exposure, they were submitted, after an interval of a week, to a second, higher concentration of hydrogen cyanide. This was calculated to kill about 70 % of the beetles, which were then treated exactly as before.

The offspring of the eighty-four parents could then be divided into five lots:

(1) Both parents killed by the low concentration.

(2) Both parents survived the low concentration but were killed by the high one.

(3) Both parents survived both concentrations.

(4) One parent killed by the low concentration.

(5) Both parents survived the low concentration and one killed by the high one.

The last two groups were destroyed, leaving three groups whose parents had different degrees of resistance. There were five cultures in each group. Those in the first were labelled A, B, C, D, E; in the second, F, G, H, I, J; and in the third, K, L, M, N, O. Each of these is referred to as a family. As the first filial generation pupated, the pupae were removed at intervals and sorted into males and females. When these became adult, brother-sister cultures were made up daily until there were twelve of these from each of the original five cultures in each of the three groups, i.e. there were sixty pairs in each group. These were allowed to reproduce as before, and at the age of 17 days they were exposed to a certain concentration of hydrogen cyanide and the percentage kill in each family obtained. The same technique that was employed to deal with the parents was used also in this generation, except that they were only exposed to one concentration. In each fumigation, of about twelve pairs of beetles, there were representatives of each group and of as many families as possible. This mixing up of the members of different families instead of fumigating only the members of one family at one time eliminated or exposed any unforeseen factors which might have prejudiced the result. The mean concentrations for all fumigations in which there were representatives of any family have been calculated and are recorded in Table III. It will be seen that there is very little difference between these mean concentrations, although in the actual fumigations

the concentration varied from 0·57 to 0·62 mg./l. The results of any fumigations in which the concentration was outside this range, were neglected. Certain other experiments also had to be neglected as they gave an unexpectedly low kill. It has been mentioned that it was usual to perform two fumigations per day, one in the morning and the other in the afternoon. After several fumigations had been carried out it was noticed that the afternoon kill was considerably lower than the morning kill, the latter being about what was expected. Table II gives the results of morning (M.) and afternoon (A.) fumigations.

Table II

	Concentration of hydrogen cyanide mg./l.	No. fumigated	No. killed
M.	0·59	24	21
M.	0·50	22	14
A.	0·50	24	4
M.	0·59	24	13
A.	0·56	22	1
M.	0·60	24	12
A.	0·60	24	3

The only difference between the two lots was that, while in the morning the beetles were removed from their culture about half an hour before the commencement of the fumigation, the beetles for the afternoon fumigation were removed from their cultures about 1.0 p.m. and left in tubes without any flour during the lunch interval. They could then be tipped into their fumigation cages after lunch and the fumigation commenced almost immediately. This meant that about $1\frac{1}{2}$ hr. elapsed between the removal of the insects from flour and the commencement of the fumigation. This process was therefore reversed, so that in the morning there was a long interval, and in the afternoon a short one, with the following result:

	Concentration of hydrogen cyanide mg./l.	No. fumigated	No. killed
M.	0·59	24	0
A.	0·62	24	14

The short interval was thereupon taken as the standard and the remaining results were quite regular. This phenomenon is discussed, together with the results of some further experiments, in § 6 of this paper.

Table III gives the results of this experiment. The mean percentage kill and its standard errors are given for each group. The differences between groups 1 and 3 and 2 and 3 are significant but not the difference between groups 1 and 2.

Table III

Parent	No.	No. killed	%	Mean concentration mg./l.
Group 1. Both parents killed by a low concentration				
A	14	12	85·7	0·589
B	16	14	87·5	0·581
C	12	6	50·0	0·598
D	14	7	50·0	0·581
E	16	9	56·3	0·584
Mean % kill = 65·9 ± 8·68. Mean concentration = 0·587.				
Group 2. Both parents survived low concentration and killed by high concentration				
F	10	4	40·0	0·592
G	10	8	80·0	0·592
H	14	9	64·3	0·587
I	12	7	58·3	0·590
J	16	8	50·0	0·592
Mean % kill = 58·5 ± 6·75. Mean concentration = 0·591.				
Group 3. Both parents survived both concentrations				
K	18	4	22·2	0·587
L	14	3	21·4	0·587
M	14	8	57·1	0·589
N	20	7	35·0	0·576
O	16	5	31·3	0·597
Mean % kill = 33·4 ± 6·47. Mean concentration = 0·587.				

As a further test, two fumigations were carried out in which about sixty adults from various families in groups 1 and 3 were exposed to the same concentrations of cyanide.

Concentration mg./l.	Group	No. fumigated	Percentage killed
0·64	1	55	82
0·64	3	60	43
0·59	1	52	54
0·59	3	60	37

The males and females were not sorted out in this test, and the high percentage kill in group 1 in the first test may be due to an unusually high proportion of females which are more susceptible than males.

Accurate work was difficult for two reasons:

(1) The great loss of gas during the course of the fumigation (see § 7).

(2) The difference in susceptibility of males and females being of the same order as the difference between the same sexes of two different groups.

On referring back to notes on the original fumigation of the parents, the same difference between morning and afternoon fumigations was observed. It is therefore clear that certain pairs of beetles were included

in group 2, which, had they been fumigated under exactly the same conditions as the others, might have come in groups 1, 4 or 5. It was then found that all the families in group 2 were descended from parents that had been fumigated in the afternoon. Group 2 was therefore artificial, and it is interesting to note that it was not significantly different from group 1. As the second series of fumigations, by which group 3 was defined, were all morning ones, this group was quite distinct from the other two. Group 2 was therefore eliminated from experiments on further generations. In order to reduce the numbers of insects to be handled, only the two most resistant, and the two most susceptible families were bred from. Those selected were A and B in group 1, and K and L in group 3. The pupae of the second filial generation of these families were removed from their cultures at intervals, and, as the adults emerged, fresh cultures of each family were set up about every other day. There were about forty to sixty beetles in each culture. When these adults had laid eggs and were from 12 to 14 days old, they were fumigated and dosage-mortality curves obtained from each family, using the same technique as has been described above for the different stages.

Experiments were not performed on the third, fourth, and fifth filial generations, but the stocks were maintained. Each family was kept down to about 1000 individuals in each generation. To avoid any unconscious selection in doing this, the insects which pupated in a given period were retained and the others destroyed. When the sixth filial generation was pupating, cultures were made up exactly as described above for the second filial generation. In the sixth and seventh filial generations about 100 beetles were available for each test and about ten tests were performed on each family. The second apparatus, which had two insect chambers, was used for these experiments. It was thus possible to carry out tests on two families simultaneously.

The results of all these experiments are shown in the concentration/percentage mortality graphs in Text-fig. 9 and log concentration/probit graphs in Text-fig. 10. The limits of error of the concentrations required to give a 50 % kill have been calculated for the males and females, and these concentrations are shown in Table IV, and diagrammatically in Text-fig. 5. Only the 50 % points are shown for families A and K of the males, and K of the females in the second filial generation, as the data were such that a negative result was obtained for the factor $b^2 - t^2\,(Vb)$ of Bliss's equation, i.e. the slope of the regression line does not differ significantly from the horizontal.

The high error in the estimate of the 50 % kill of females of the B and

L families of the F_2 generation is due to the fact that most of the kills were over 50 % and that the slope of the regression line was very low. For reasons described in § 8 the result of tests on females of the F_6 generation in family A are not given here.

Table IV. *Summary of data of inheritance experiments*

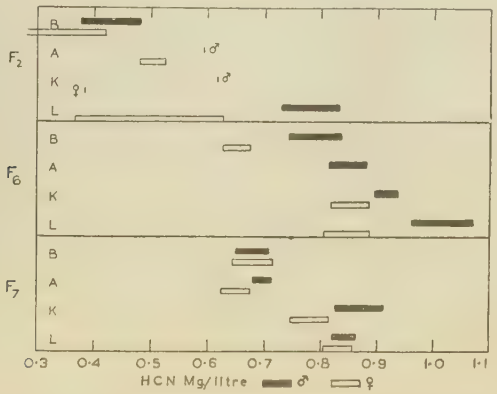
Family	Generation	n	χ^2	50 % kill mg./l.
		Males		
A	F_2	3	7·2158	0·592
B	F_2	5	9·6656	0·426 ± 0·047
K	F_2	3	5·9311	0·617
L	F_2	4	1·2827	0·782 ± 0·049
A	F_6	6	5·3119	0·846 ± 0·034
B	F_6	8	12·0418	0·780 ± 0·054
K	F_6	7	18·7751 (S.)	0·919 ± 0·024
L	F_6	8	7·1134	1·031 ± 0·036
A	F_7	16	74·9525 (S.)	0·696 ± 0·017
B	F_7	10	55·6249 (S.)	0·679 ± 0·028
K	F_7	9	107·5925 (S.)	0·867 ± 0·042
L	F_7	10	10·6023	0·840 ± 0·019
		Females		
A	F_2	3	0·4548	0·502 ± 0·024
B	F_2	6	18·2383 (S.)	0·356 ± 0·197
K	F_2	3	4·4834	0·384
L	F_2	4	2·5454	0·553 ± 0·186
A	F_6	—	—	—
B	F_6	8	31·0979 (S.)	0·650 ± 0·025
K	F_6	7	5·2966	0·851 ± 0·034
L	F_6	8	69·6269 (S.)	0·845 ± 0·041
A	F_7	16	39·3113 (S.)	0·649 ± 0·027
B	F_7	10	107·4017 (S.)	0·678 ± 0·036
K	F_7	9	10·3129	0·781 ± 0·034
L	F_7	10	14·9025	0·829 ± 0·027

Where the data depart significantly from the straight line that has been fitted to them, the value of χ^2 is followed by S. The limits of error above and below the 50 % kill value are not necessarily the same, but for convenience the larger error is given.

It will be seen from Text-fig. 5 that there is a considerable difference in resistance from generation to generation. The different apparatus may account for the difference between the second and sixth filial generations; as has been explained above, there was a large surface of mercury exposed in the first apparatus and the vapour was found to be highly toxic to insect eggs. Eggs of both *Tribolium* and the bed-bug were killed by exposure to mercury vapour for less than 24 hr. at 27 and 23° C. respectively, whereas other stages of these insects were unaffected under the same conditions. It would be surprising if the greater surface of mercury exposed had such a marked additional toxic effect upon adults which were apparently unaffected after a week's exposure to mercury

vapour. Other references to this have been recently recorded by Gough (1938*b*).

There seemed to be no obvious reason for the difference between the sixth and seventh filial generations. Shepard *et al.* (1937) were able to reproduce after an interval of time their original results of fumigation experiments on *Tribolium*. Their data were also in agreement with those of other workers. On the other hand, with other fumigants and test insects their results were more variable. Tattersfield & Martin (1935) have stressed the importance of carrying out comparative toxicity tests at not too widely separated intervals of time, though they were more particularly concerned with plant feeding insects.



Text-fig. 5. Limits of error of concentrations of hydrogen cyanide required to give a 50 % kill of different families of beetles (B, A, K, L) in different generations (F_2, F_6, F_7).

Even more surprising is the difference in the relative resistance of males and females in the different generations. It is quite evident from either Text-fig. 10 or Table IV that in the second filial generation the females are much more susceptible than the males, whereas in the seventh filial generation, the resistance of the two sexes is about the same. It is possible that as normally the female appears to be more susceptible than the male, the original fumigation of the parents tended to select the more resistant females. If this were so, it is difficult to understand why this increase in resistance of the females was not observed in the second filial generation.

Throughout the series there is, however, a distinct significant difference between the males of the resistant and of the susceptible groups, especially between families B and L. In both the second and the

sixth filial generations the order of resistance is the same, i.e. B, A, K, L, and in both generations the A and K families are very close together, although in the seventh filial generation these two families are as widely different as the other two. In the females the difference between the two groups is significant in the sixth and seventh generations, though in the second generation the 50 % kills for A are higher, and for K lower than would have been expected. This evidence of the existence of the inheritance of resistance towards certain poisons obviously has a very important bearing on all chemical methods of insect control. It is, perhaps, likely to be of less importance in plant protection, where the entomologist is more concerned in reducing the numbers of a pest to a certain economic limit, than in the fumigation of stored products, where a complete kill is the aim. In the former type of control vast numbers of insects escape for purely accidental reasons and not necessarily because they are more resistant. These insects might keep the resistance of the insect population to a given poison at its normal level. In a well-conducted fumigation the most likely reason for insects surviving is that they are highly resistant to the fumigant, and if these insects alone are allowed to reinfect the same product the population derived from them might have a considerably higher level of resistance than normal. It would be interesting to know if an insect which is highly resistant to a certain poison in one stage, is also more resistant in other stages, and also if it is more resistant to other poisons.

Correlation of resistance with other characters

Having obtained families of beetles with significantly differing degrees of resistance to hydrogen cyanide an attempt was made to correlate this with other characters. There was no superficial morphological difference, nor was there much difference in the rates of development as measured by length of life cycle, though family K appeared to take a few days longer than any of the others. This slight lengthening of life cycle apparently occurred in the larval stage.

There did not seem to be any simple relationship between body size, as measured by live weight, and resistance:

Wt. of 100 beetles, F_7

B (most susceptible to cyanide)	A	K	L (most resistant to cyanide)
0·2114 g.	0·1872 g.	0·1939 g.	0·2114 g.

Mr G. V. B. Herford carried out respiration experiments on different families in a modified Barcroft respirometer. The following are the results

expressed as the oxygen uptake per mg. live weight of beetle in c.mm./hr. at 27° C. 100 beetles were used in each experiment:

B (most susceptible to cyanide)	A	K	L (most resistant to cyanide)
2·163	2·422	1·925	2·803

If these experiments can be considered representative they suggest that a difference in rate of metabolism between individuals is not necessarily correlated with a difference of resistance, as L has the highest respiratory rate and yet is the most resistant family. B has a lower respiratory rate than any of the others except K, and yet is the most susceptible to hydrogen cyanide. As these respiration experiments were not repeated and other respiration measurements showed considerable variation, they cannot be taken as evidence against the validity of Cotton's theory. It must, however, be regarded as possible that respiratory rate is not necessarily the only factor which determines resistance, even within one stage of an insect. The relationship of resistance to metabolism between different individuals of one stage must, with our present knowledge at any rate, be considered separately from the relationship of resistance to metabolism between the different stages of one insect.

6. Effect on resistance to hydrogen cyanide of the time elapsing between removal of the beetles from flour and their fumigation

It was not possible to investigate this question as fully as was desired, so that the results are only comparative and qualitative.

A number of cultures of adult beetles were made up and left in the constant temperature and humidity room. After some days the beetles were removed from the flour at certain specified times to empty jars. This operation was carried out in the constant temperature room. The beetles were then exposed to the same concentration of hydrogen cyanide for $1\frac{1}{2}$ hr. and afterwards the males and females sorted and the percentage kill obtained. The results are shown in Table V.

It will be seen from these tables that there is considerable variation in the results of the three experiments. There was some difference in the age of the three lots. Also the beetles in Exp. 3 were left in flour for a much longer period before the experiment was carried out. In spite of the variation, certain facts emerge.

Table V

No. of hours previously removed from flour	Concentration mg./l.	Males: No. fumigated	Males: Recoveries 1st day after fumigation	Males: Recoveries 2nd day after fumigation	Males: % kill	Females: No. fumigated	Females: Recoveries 1st day after fumigation	Females: Recoveries 2nd day after fumigation	Females: % kill
				Experiment 1					
24	1·07	40	2	1	63	49	9	4	41
6	1·06	32	1	0	65	61	11	8	25
3	1·07	50	1	1	78	66	9	1	42
1	1·06	36	0	0	80	52	2	5	65
½	1·05	40	0	0	82	52	2	6	71
0	1·05	36	0	0	86	54	1	0	89
				Experiment 2					
20	1·01	79	4	4	67	44	9	8	41
15	1·01	76	8	3	74	59	10	7	48
6	0·99	75	2	0	59	73	9	7	40
3	0·99	70	2	2	64	84	5	1	31
1	0·97	70	3	1	61	61	3	9	54
0	0·97	64	4	1	69	74	2	1	92
				Experiment 3					
12	1·66	54	1	5	61	61	1	7	36
8	1·66	55	2	7	55	57	0	7	37
8	1·73	50	3	0	54	59	0	1	37
4	1·79	47	1	0	74	64	0	2	48
2	1·81	50	1	1	68	48	1	0	44
0	1·79	45	0	0	82	49	1	0	76

(1) The males are less affected by early removal from flour than are the females. The following are the lowest and highest kills of the two sexes in each experiment:

Exp.	Males %	Females %
1	63–86	25–89
2	59–74	31–92
3	54–82	34–76

(2) It seems that there are rather more recoveries of apparently dead beetles afterwards among the females than among the males. There are also more recoveries in both sexes in those insects which have been out of flour for the longer periods. This suggests that lack of flour tends to produce a greater degree of stupefaction. This might account for the greater resistance of these beetles by a "protective stupefaction" effect as described by Pratt *et al.* (1931).

There are several possible explanations.

(i) *Effect of mechanical shock*

The actual mechanical shock of being removed from flour might affect the insects merely by making them more active, and possibly more susceptible. This seemed unlikely for two reasons:

(*a*) All beetles were disturbed to some extent during transference from the empty jar to the fumigation cage, and the introduction of that into the insect chamber.

(*b*) If this theory were correct it might be supposed that the increase in resistance with increasing time out of flour (up to several hours in each experiment) was accounted for by the beetles taking up to that number of hours to settle down. Actually, as far as could be observed, they settled

Table VI

	No. of hours previously removed from flour		% kill	
			Males	Females
(1)	8	Not shaken	54	37
	8	Shaken	67	34
	2	Not shaken	68	44
	2	Shaken	84	61
	0	—	82	76
(2)	2	Not shaken	30	49
	2	Shaken	56	53
(3)	2	Not shaken	53	44
	2	Shaken	41	43

down within about 15 min. of being removed. It is possible, however, that the effect of activity lasts for a greater period than the activity itself.

Experiments were carried out in which two lots of insects were removed from flour at a certain time and placed in their fumigation cages. Immediately before fumigation the one lot was treated exactly as it would have been had it just been removed from flour, i.e. the beetles were shaken in a sieve, removed from this by tapping on to a piece of paper, brushed into a tube with a fine brush, and finally tipped into the cage again and fumigated with the other lot which was placed at the same time as gently as possible in the fumigation chamber. The results are shown in Table VI.

Though, once again, these results are conflicting it seems that this is at least one of the factors concerned. It may operate through the production of a volatile substance emitted by the adults, under certain conditions (see § 7).

(ii) *Effect of contact with flour*

It is possible that mere contact with flour has some effect on the insects, perhaps on the tone of the muscles, or the respiratory rate or the degree to which the spiracles are opened. Though no experiments were performed, this possibility might be tested by placing the beetles in some non-toxic and inert substance similar in texture to flour, and seeing if removal from that substance at various times affected their resistance.

(iii) *Amount of food in the gut*

If the insect is feeding practically all the time, the amount of food present in some part of the gut may affect its resistance directly or indirectly. It seems unlikely that such a short period as an hour would have so great an effect as was observed but, on the other hand, it is interesting to compare the similarity in reaction to hydrogen cyanide of 0–1-day-old adults and older adults which have been removed from flour some time previously. As has been mentioned, Lindgren (1935) found a great difference between the rate of metabolism of old adults in flour and out of flour. Young adults, by which he meant those less than 4 days old, did not exhibit this difference, and had about the same rate of metabolism as older adults in flour. Lindgren suggests that the young adults do not feed for some days after emergence. If this could definitely be demonstrated it would at least suggest that the amount of food in the gut had some influence on resistance.

Craufurd-Benson (1938) obtained more uniform results in toxicity tests of Derris preparations to *Ahasverus advena* Waltl. when the insect was removed from its food medium 24 hr. before test, than when insects freshly removed were tested. He suggests that this period of starvation ensures uniformity of metabolic processes.

7. Emanation of a self-toxic and volatile substance by the adults

(i) *Emanation of a self-toxic substance*

During early tests a muslin bag was used to contain the insects. This was not very satisfactory as it absorbed hydrogen cyanide, and it was not easy to insert and extract insects, especially the adults. Another type of container used was a short length of glass tubing about 1 cm. long and 0·5 cm. internal diameter. This was closed at each end with bolting silk secured with fine iron wire. At the end of the first test of adult

beetles in this type of container all the controls were found to be dead or moribund. The latter would react to stimulation by twitching and some were sufficiently lively to attempt to walk when placed right side up but had difficulty in co-ordinating their movements. A series of experiments was then carried out with various numbers of adults of different ages enclosed in this container. All these experiments were carried out in the constant temperature room (see Table VII).

Table VII

Sex	Whether mated (M.) or virgin (V.)	Age weeks	No. of hours in container	No. of beetles	At end of experiment: No. normal	No. twitching	No. motionless	% affected
Male	M.	2	19	42	42	0	0	0
Male	M.	2	19	40	40	0	0	0
Female	M.	2	19	41	41	0	0	0
Female	M.	2	19	47	47	0	0	0
Male and female	M.	4	2	60	60	0	0	0
Male and female	M.	4	16	55	55	0	0	0
Male	M.	5	2	73	11	9	53	85
Female	M.	5	2	59	12	39	8	80
Male	V.	6	2	61	53	4	4	13
Male	V.	6	16	60	55	2	0	3
Male	V.	6	16	49	48	1	0	2
Female	V.	6	2	60	0	4	56	100
Female	V.	6	2	30	2	6	22	93
Female	V.	6	2	15	0	11	4	100
Female	V.	6	2	5	0	0	0	0
Female	V.	6	20	5	4	1	0	—

It appeared from these experiments that from about the age of 4–5 weeks the adults, under certain conditions, emitted some substance which was self-toxic and, furthermore, that while mated adults of both sexes produced this substance, virgin females produced more than virgin males. That the higher mortality among these females was not due to greater susceptibility was shown by the fact that males placed in contact with them were equally affected.

(ii) *Volatility of the self-toxic substance*

The following experiments suggested that this self-toxic substance was volatile:

(1) Two containers were placed in line with one another and were only separated by muslin. Adults which produced this emanation were placed on one side and adults which apparently did not produce it on the other side. The latter were affected, showing that the emanation had diffused from one container to the other. Similar results were obtained

if the second type of adults were suspended in a muslin bag over a number of the emanation-producing adults, all in a closed tube.

(2) Beetles placed in a container, through which a slow stream of air was drawn, were unaffected, suggesting that the substance was removable as a gas by convection. Under such conditions, however, they might not be stimulated to produce the substance.

(3) Beetles placed in a muslin bag were not affected. Under these conditions there is sufficient opportunity for the substance to escape by diffusion and convection.

As some experiments suggested that this substance was produced in much greater amounts by virgin females than by any other type of adult, it was thought that it might be an aphrodisiac. An experiment was performed in which a number of males and a number of females were enclosed at the two ends of the branches of a Y tube. Virgin males were then admitted at the base of the Y. Practically equal numbers went in each direction.

Effect of high humidity and high concentrations of carbon dioxide.

In order to eliminate these two obvious possibilities the following experiments were carried out. In the first, small numbers of beetles were placed in a tube over water. None of these was affected. In the second, a tube with a large number of beetles was surrounded by soda lime: forty-eight out of fifty of these beetles were affected within 2 hr. It was noticed that the soda lime near each muslin-covered end of the tube had turned a dull green. It was then found that if a small flake of sodium or potassium hydroxide were held over a number of adult beetles a green coloration developed on the surface of the flake. The speed with which this occurred, depended on the number of insects and the period during which they had been enclosed. If the sodium hydroxide was left for several hours near the beetles it ultimately turned a rusty brown colour.

Whether this substance was actually given off as a gas or, as seems more likely, as a liquid secretion which subsequently volatilized is not known. It may be emitted by special glands or might even be given off from the faeces. In tubes in which the insects had been kept the muslin and the sides of the tube were usually discoloured.

(iii) *Absorption of hydrogen cyanide by a volatile substance evolved by the adults*

It has been explained that a gas sample was taken at the beginning and end of each test. Usually there was a slight loss of gas during the course of the test due to absorption by the insects, their container, stop-

cock grease, etc. In a series of twenty tests on eggs, larvae, pupae and freshly emerged adults, the average loss per test was 0·037 mg./l., ranging from 0 to 0·06 mg./l. A series of tests on 28-day-old adults then gave the following losses in order:

0·07, 0·10, 0·09, 0·16, 0·18, 0·21 mg./l.

A test was then performed without any insects and there was a loss of 0·18 mg./l. Flask *X* was then isolated, and gave a loss of 0·05 mg. l. It was thought possible that some spray from the solution of potassium hydroxide, which was used for conditioning the air, might have been drawn into the apparatus, so this was dismantled and cleaned. The mercury was washed with dilute nitric acid followed by distilled water. All rubber connexions were renewed and all glass parts cleaned with nitric cleaning mixture. The apparatus was then reassembled. Sulphuric acid solution was used for conditioning the air, and an extra spray trap was also inserted. Forty-six tests were then carried out on eggs, larvae and pupae and the loss of hydrogen cyanide never exceeded 0·04 mg./l., the average being just over 0·02 mg. l. Then eight tests on adults were performed and the average loss was 0·07, ranging from 0·06 to 0·16 mg./l. Only fairly small numbers of adults were used in each of these tests. Nine tests on stages other than adults then gave an average loss of 0·08 mg./l., and the next eleven tests, also on other stages, gave an average loss of 0·04 ranging from 0·03 to 0·07 mg./l. Then eight more lots of adults were fumigated with the following losses: 0·12, 0·16, 0·21, 0·21, 0·17, 0·26, 0·30, 0·30 mg./l. The last losses each represented about 40 % of the original concentration of hydrogen cyanide in those tests. The apparatus was then cleaned out again and similar results obtained, i.e. with all stages but adults the loss was practically negligible, but when adults were used the loss became very high. Moreover, the effect was cumulative, that is, the loss of cyanide increased each time adults were used, and was noticeable for some time after adults had been used. This suggested that the adults give off some volatile substance which combines loosely with hydrogen cyanide under certain conditions. That the loss is due merely to a greater absorption by the adults is ruled out by the fact that freshly emerged adults do not cause it, and by the fact that it is residual and occurs also after the insects have been removed. As the apparatus was evacuated twice between each test, it must be assumed that this substance either condenses out again on the internal surfaces of the apparatus or, very improbably, combines in some way with either the glass or the mercury. As the lower ends of the large vessels *X* and *Y* were

possibly at a slightly lower temperature than the upper ends it may be that condensation tended to occur there. Probably at the reduced pressure during evacuation the compound containing hydrogen cyanide dissociated and the hydrogen cyanide itself was removed, leaving the substance on the walls of the glass vessels. It is remarkable that the repeated evacuation failed to vaporize it. It was also unaffected by a high concentration of cyanide. In order to make use of tests on adults an arbitrary concentration had to be selected. As the concentration, taken every 20 min., plotted against time was found to give a straight line, it was assumed that the mean of the first and final gas samples would give a fairly good idea of the effective concentration.

How far this substance can be identified with the one which was self-toxic it is difficult to say. In all the tests on adults, wire-gauze cages were used and the controls were quite unaffected. It may perhaps be assumed that this substance is being emitted all the time, and in the muslin-capped tubes it was not removed sufficiently quickly by diffusion. 14-day-old adults were, however, unaffected by being enclosed in the muslin-capped tubes, but they certainly caused as great a loss of cyanide as the older adults. The difficulty of carrying out analytical tests on this substance is emphasized by the fact that the greatest loss of cyanide was about 0·3 mg./l. or a total loss of about 0·00165 g. Presumably the substance concerned would be present in an amount of the same order.

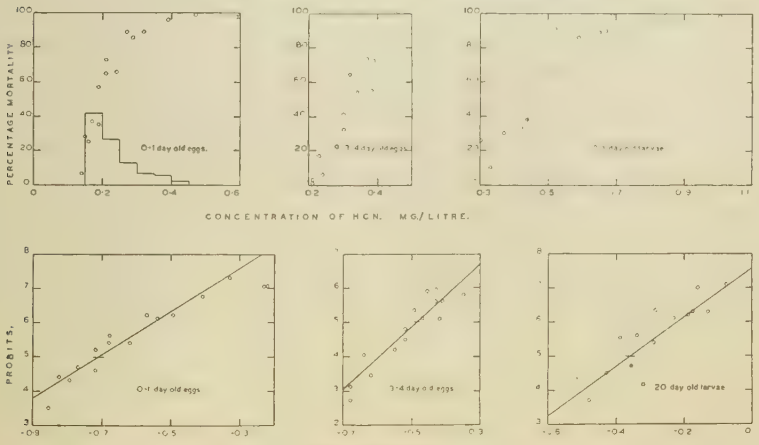
(iv) *Other references to emanations of* Tribolium

These emanations may also be associated with Park's (1935) work on the conditioning of flour by *Tribolium*, and possibly with the peculiar smell of this insect. Chapman (1926) mentions that a gas is given off by adults of *Tribolium* when irritated. When larvae and pupae were enclosed with a number of beetles which had been irritated, about 10 % of these larvae and pupae gave rise to monstrosities. Mature larvae emerged with a combination of larval and pupal structures and pupae gave rise to deformed adults. Chapman associates these abnormalities with other reports of abnormal metamorphosis in insects.

It seems, therefore, that a study of this emanation of *Tribolium* might lead to very interesting and important results, and the brief observations described here may suggest lines along which the chemical constitution of this emanation may be investigated.

8. The dosage-mortality curve

Text-figs. 6–10 are graphs expressing the relationship between concentration of hydrogen cyanide and percentage mortality, or log concentration and mortality converted to probits. As the points were so scattered no attempt was made to fit the concentration-mortality curves. Only the graph of 0–1-day-old eggs in Text-fig. 6 shows anything like a smooth curve and the histogram on the same graph has been derived from this curve by plotting differences in percentage kill for regular dose



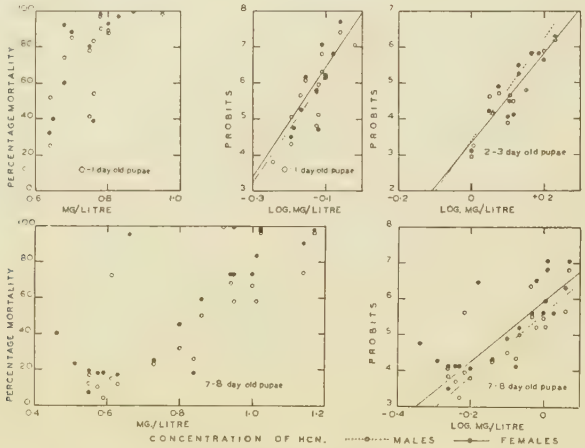
Text-fig. 6. Dosage-mortality graphs for eggs and larvae.

intervals (0·05 mg./l. here) against the mid-point of that dose interval. This method is described by Trevan (1927). The histogram exhibits the usual type of asymmetry though it is exceptionally well marked. This is partly due to the allowance for the failure of a certain percentage of the controls to hatch, and possibly to the great error in the estimation of hydrogen cyanide at the very low concentrations.

As might be expected from the irregularity of these curves, the values for χ^2 of the regression line obtained by Bliss's method are all very high. Out of fifteen regression lines of different stages of *Tribolium*, twelve diverged significantly from a straight line. It is interesting to note that the remaining three were experiments in which fewer tests were carried out. In the inheritance experiments nine out of twenty-four regression lines diverged significantly from the straight line fitted to them.

Craufurd-Benson (1938) obtained erratic results in toxicity tests of Derris preparations to *Ahasverus advena* Waltl. until he standardized his technique very rigidly. In the present work, as temperature and humidity, length of exposure, gas concentration and circulation, were all controlled it is difficult to account for the great variation observed in the results. The following are possible suggestions:

(1) The typical dosage-mortality curve is not necessarily applicable to the reaction of all organisms to all poisons. In view of the frequency with which this type of curve is obtained when the percentage of a number

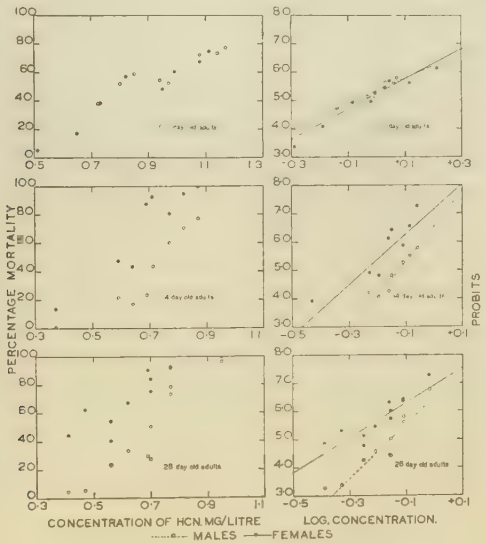


Text-fig. 7. Dosage-mortality graphs for pupae.

of organisms reacting in a certain way to a certain stimulus, is plotted against a function of the magnitude of that stimulus, this suggestion is scarcely acceptable. Strand (1930), and many other workers since, have used *Tribolium confusum* as a test insect and have obtained normal curves. Bliss (1935*a*) has analysed Strand's data and found them consistent with a straight regression line, when converted to probits and log concentrations.

It is also a difficult matter to decide whether or not to include points which are nowhere near any others, such as the two in the top left-hand corner of the graph of 7–8-day-old pupae in Text-fig. 7. Are these accounted for by random sampling, or is it justifiable to assume that something went wrong in those particular tests? Trevan (1927) has stated: "One thing should be insisted on, however, with great force, and that is,

that unexpected or 'discrepant' results must not be eliminated from the final average, except on the clearest evidence of a mistake in the technique." This rule has been followed in the present work. In the experiment on 2–3-day-old pupae (Text-fig. 4), it was thought reasonable to consider the tests on cultures indicated by a cross, distinct from the others, and only the latter were used when the results were treated with Bliss's method. On the other hand, odd points as those mentioned above have always been included in the calculations.

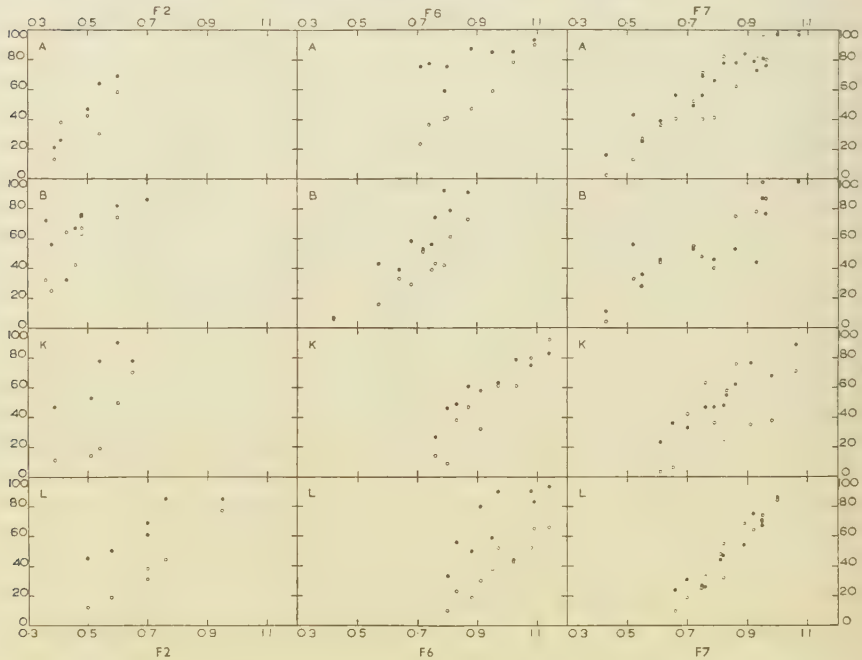


Text-fig. 8. Dosage-mortality graphs for adults.

Had the majority of the tests given consistent results it might have been reasonable to have neglected the results of "discrepant" tests, but under the circumstances it was thought best to include them all.

Several of the curves, especially those for larvae (Text-fig. 6), for 7–8-day-old pupae (Text-fig. 7) and certain of the curves shown in Text-figs. 9 and 10 exhibit, in places, a tendency to show no increase in mortality with an increase in dose. This might cast doubt on the accuracy of the estimation of the gas concentration, but as the two samples taken in each test were almost invariably similar, it may fairly be assumed that the method of gas sampling was satisfactory. If, to account for these "step-curves", one assumes that there are well-defined degrees of resistance to

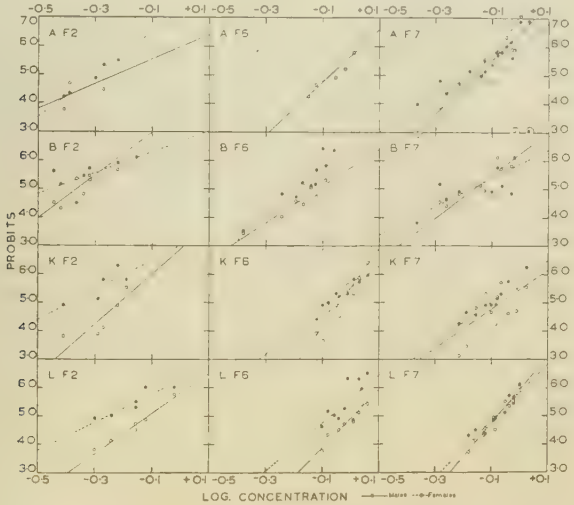
hydrogen cyanide in *Tribolium*, it would be expected that after inbreeding for several generations as had been done, this "step" effect would disappear. The curves for the A, B, and K families in the seventh filial generation (Text-figs. 9, 10) show that this is not so. In the seventh filial generation of family A this "step" effect is very marked. An exceptionally large number of tests were performed in this experiment



Text-fig. 9. Percentage mortality-concentration graphs for 3 generations F_2, F_6 and F_7 of 4 different families A, B, K, L of *Tribolium confusum*.

since in the previous generation all the points for females, with one exception, lay in the 80–90 % region, whereas the points for the males at the same concentrations ranged from 20 % upwards. As a number of dead beetles had been found in these cultures it was thought that the females might have been suffering from some disease which killed some of the beetles and lowered the resistance to hydrogen cyanide of the others. The dead insects were therefore examined, and it was found that they were mostly males. Hence, it was desired to see if a similar curve for

females would be obtained in the next generation. Though this was not found, there was a tendency for the curve to flatten out in the 80 % kill region. In the curve for family B in this generation there appears to be a horizontal portion about the 50 % kill region. Without performing very large numbers of tests under very carefully controlled conditions it would be difficult to decide whether the peculiar shape of these curves was real or not. The fact that the Slough stocks of *T. confusum*, from which the author's experimental insects were derived, had been inbred for many



Text-fig. 10. Probit-log concentration graphs for 3 generations F_2, F_6 and F_7 of 4 different families A, B, K, L of *Tribolium confusum*.

years and must have been fairly homogeneous, suggests that one must look elsewhere for an explanation of these curves.

(2) Trevan (1927) came to the conclusion that a group of about thirty animals was near the optimum size for the determination of an average lethal dose, and a significant increase in accuracy above that given by this group number is only obtained by the use of greatly increasing numbers of animals. As, nearly always, larger numbers than this were used in each test, it seems unlikely that insufficient numbers of insects were used to give accurate results.

(3) Perhaps the most likely explanation of the variable results is the short exposure period of $1\frac{1}{2}$ hr. Most other workers have used longer

periods, 5 hr. being most usual. This was tried in the first place, but the great susceptibility of *Tribolium* to hydrogen cyanide made dosing difficult and estimation of the low gas concentrations necessary was very inaccurate. Furthermore, it was desirable to be able to perform two or three tests per day.

In a short exposure the two following factors would probably have a much greater influence on the final reaction of an insect to a poison than in a longer exposure.

(*a*) *The effect of handling.* It is practically impossible to carry out such tests as these without handling the insect to some extent. The experiments described in § 6 show that the treatment *Tribolium* receives before being fumigated greatly affects its reaction. It seems unlikely, however, that disturbance due to handling would affect eggs and pupae as much as larvae and adults, whereas, in fact, all stages exhibited equally great variability.

(*b*) *Initial reaction of the insect when exposed to the gas.* This reaction will vary with the insect and the fumigant and the concentration of the fumigant. When introduced suddenly into a fumigant an insect may be stupefied almost immediately, it may be stimulated to greater activity, it may close its spiracles or open them wider, or other less obvious but equally important processes may occur. After this initial reaction, the insect may reach a more or less steady state, and it may be that the greater the length of this steady period, the less variable will the results be. The results of Pratt *et al.* (1931), on the "protective stupefaction" of certain scale insects when exposed to sublethal concentrations of hydrogen cyanide, indicate that the initial reaction of an insect to a fumigant is of great importance.

9. Summary

1. Two types of apparatus for exposing insects to known concentrations of toxic gases, under controlled conditions, were constructed and are described.

2. The relative resistance of different stages of the flour beetle, *Tribolium confusum* Duv., to hydrogen cyanide was determined, the results being treated by Bliss's method. The order of resistance was pupa (most resistant), adult, larva, egg. The difference in resistance at different ages was found for the pupa, adult and egg. The application to all these results of Cotton's theory of the relation of rate of metabolism and susceptibility to fumigants is discussed. It is considered that this theory might hold for stages other than the egg.

3. A series of experiments was carried out to discover whether individual resistance to hydrogen cyanide was inherited. It was found that the offspring of resistant individuals were significantly more resistant than the offspring of susceptible individuals, and that this difference was maintained over several generations.

4. A considerable increase in resistance was found to occur if adult beetles were removed from their flour about an hour, instead of immediately, prior to their fumigation. The reason for this increase was not determined, but various suggestions to account for it are discussed.

5. It was noted that under certain conditions, adult beetles emitted a volatile substance which was self-toxic, and also appeared to react with cyanide.

6. The dosage-mortality curves for all the experiments are discussed and possible reasons for their apparent abnormality put forward.

7. The experiments here described, which must only be regarded as preliminary, emphasize

(i) The complexity of the problems arising in the study of the resistance of living organisms to toxic substances.

(ii) The need for carrying out such experiments under as natural conditions as possible.

(iii) The importance of a careful analysis of factors which affect the resistance of the organism.

Thanks are due to Prof. J. W. Munro under whose direction this work was carried out at the Imperial College of Science and Technology, Biological Field Station, Slough; to Dr H. H. S. Bovingdon for supervising the construction of the first apparatus; to Mr G. V. B. Herford for assistance in the designing of the second apparatus and for carrying out respiration experiments; to Drs Richards and Page for much helpful advice and criticism; and to Dr Lubatti who took the photographs of the first apparatus.

REFERENCES

Bliss, C. I. (1935*a*). The calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.

—— (1935*b*). The comparison of dosage-mortality data. *Ann. appl. Biol.* **22**, 307.

Bodine, J. H. (1929). Factors influencing the rate of respiratory metabolism of a developing egg (Orthoptera). *Physiol. Zoöl.* **2**, 459.

Bovingdon, H. H. S. (1934). An improved laboratory apparatus for fumigation experiments. *Ann. appl. Biol.* **21**, 704.

—— (1935). The bed-bug (*Cimex lectularius* L.), Fam. Cimicidae. *Rochester Nat.* N.S., **1**, 3.

Boyce, A. M. (1928). Studies on the resistance of certain insects to hydrocyanic acid. *J. econ. Ent.* **21**, 715.

Busvine, J. R. (1938). The toxicity of ethylene oxide to *Calandra oryzae*, *C. granaria*, *Tribolium castaneum* and *Cimex lectularius*. *Ann. appl. Biol.* **25**, 605.

Burkholder, J. R. (1934). A quantitative study of respiratory metabolism in single developing eggs (Orthoptera). *Physiol. Zoöl.* **7**, 247.

Chapman, R. N. (1926). Inhibiting the process of metamorphosis in the confused flour beetle. *J. exp. Zool.* **45**, 293.

Cotton, R. T. (1932). The relation of respiratory metabolism of insects to their susceptibility to fumigants. *J. econ. Ent.* **25**, 1088.

Craufurd-Benson, H. J. (1938). A method for testing liquid contact insecticides. *Bull. ent. Res.* **29**, 41.

Fink, D. E. (1925). Metabolism during embryonic and metamorphic development of insects. *J. gen. Physiol.* **7**, 527.

Fisher, R. A. (1932). *Statistical Methods for Research Workers.* Oliver and Boyd.

Gough, H. C. (1938*a*). Use of sulphur dioxide against the bed-bug. *Nature, Lond.*, **141**, 164.

—— (1938*b*). Toxicity of mercury vapour to insects. *Nature, Lond.*, **141**, 922.

Hamlin, J. C. & Reed, W. D. (1927). Insect revival after fumigation. *J. econ. Ent.* **20**, 400.

Knight, H. (1925). Factors affecting efficiency in fumigation with hydrocyanic acid. *Hilgardia*, **1**, 35.

Lindgren, D. L. (1935). The respiration of insects in relation to the heating and fumigation of grain. *Tech. Bull. Minn. agric. exp. Sta.* **109**.

Lubatti, O. F. (1935). Determination of fumigants. III. Micro-determination of ethylene oxide and hydrogen cyanide. *J. Soc. chem. Ind., Lond.*, **54**, 424.

Melander, A. L. (1914). Can insects become resistant to sprays? *J. econ. Ent.* **7**, 167.

Meldrum, N. U. (1933). *Cellular Respiration.* Methuen.

Melvin, R. (1928). Oxygen consumption of insect eggs. *Biol. Bull. Wood's Hole*, **55**, 135.

Park, T. (1935). Studies in population physiology. IV. Some physiological effects of conditioned flour on *Tribolium confusum* Duval and its populations. *Physiol. Zoöl.* **8**, 91.

—— (1936). Studies in population physiology. V. The oxygen consumption of the flour beetle *Tribolium confusum* Duval. *J. cell. comp. Physiol.* **7**, 313.

Potter, C. (1937). A biological study of the fumigation of empty warehouses with hydrogen cyanide and ethylene oxide. *Ann. appl. Biol.* **24**, 415.

Pratt, F. S., Swain, A. F. & Eldred, D. N. (1931). A study of fumigation problems: "Protective Stupefaction," its application and limitations. *J. econ. Ent.* **24**, 1041.

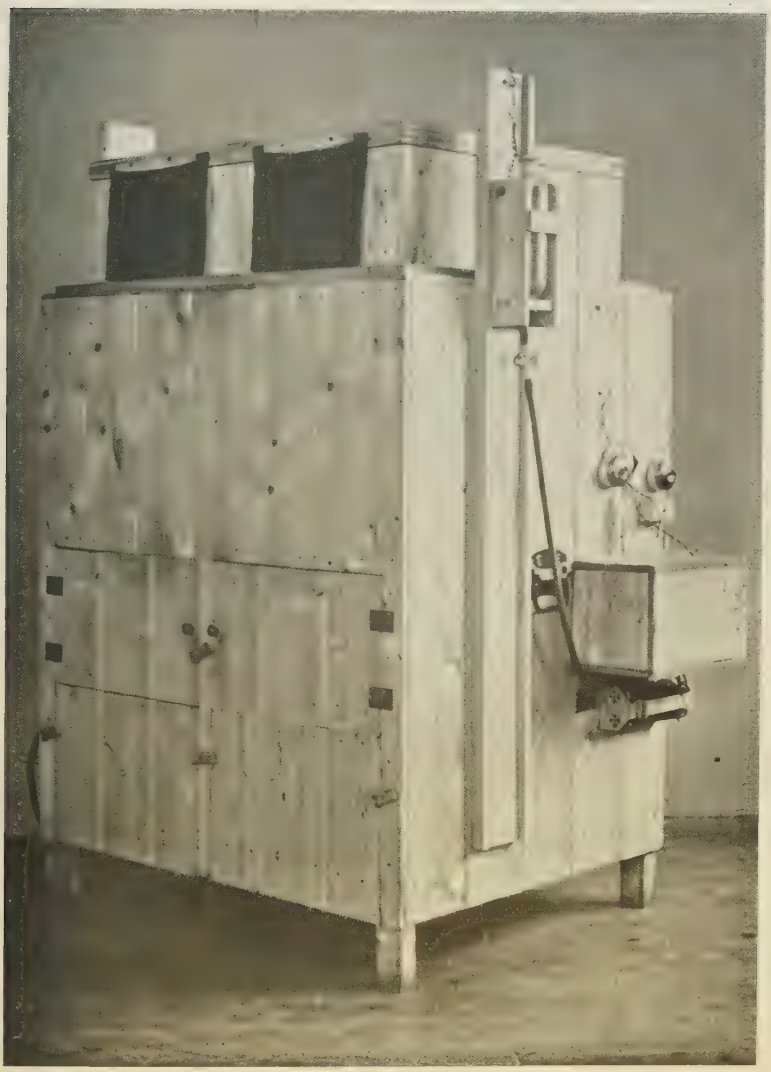
Quayle, H. J. (1922). Resistance of certain scale insects in certain localities to hydrocyanic acid fumigation. *J. econ. Ent.* **15**, 400.

Shepard, H. H., Lindgren, D. L. & Thomas, E. L. (1937). The relative toxicity of insect fumigants. *Tech. Bull. Minn. agr. exp. Sta.* **120**.

Strand, E. D. (1930). Measuring the toxicity of insect fumigants. *Industr. Engng Chem.* (Anal. Ed.), **2**, 4.

Tattersfield, F. & Martin, J. T. (1935). The problem of the evaluation of rotenone-containing plants. I. *Derris elliptica* and *Derris malaccensis*. *Ann. appl. Biol.* **22**, 578.

Trevan, J. W. (1927). The error of determination of toxicity. *Proc. roy. Soc.* B., **101**, 483.



GOUGH.—Factors affecting the Resistance of the Flour Beetle, *Tribolium confusum* Duv., to Hydrogen Cyanide (pp. 533–571)

Uchida, H. (1938). Relation of effectiveness of the fumigation by carbon bisulphide to the development of the egg, larva and pupa of *Chaetodacus dorsalis* Hendel. Prelim. Rep. (in Japanese), Abstract from *Rev. appl. Ent.* A, **26**, 263.

Wigglesworth, V. B. (1934). *Insect Physiology*. Methuen. p. 78.

Woglum, R. S. (1925). Observations on insects developing immunity to insecticides. *J. econ. Ent.* **18**, 593.

EXPLANATION OF PLATES XXXI AND XXXII

Photographs of first apparatus

Plate XXXI. Apparatus closed.

Plate XXXII. Apparatus with top removed.

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INVESTIGATIONS UPON THE PEA STRAIN OF *HETERODERA SCHACHTII* SCHMIDT AND ITS RÔLE IN THE CAUSATION OF PEA SICKNESS

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(With Plates XXXIII and XXXIV)

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1. Introduction

Among the nematodes of the family Anguillulinidae attacking the roots of plants of economic importance the genus *Heterodera* contains two of the most important species, namely, *H. marioni* (Cornu, 1879; Goodey, 1932), and *H. schachtii* (Schmidt, 1871). The latter species was discovered by Schacht (1859) infesting the roots of sugar beet when it was found responsible for the condition known as beet sickness (Rübenmudigkeit). Kühn (1874) reported a strain which has been discovered to attack oats, and, to a lesser degree, barley, wheat and rye. Other strains have been found to attack potatoes, hops and leguminous plants.

The last strain was first found attacking peas by Liebscher (1890), who concluded that it was a different species from the beet eelworm and named it *H. göttingiana*, now regarded as a synonym of *H. schachtii*. This pea race was later found to be associated with the fungus *Fusarium vasinfectum* Atk. var. *Pisi* van Hall (= *Fusarium oxysporum* Schl. *f.* 8

Snyder) by Capus (1917, 1918) in France. In Britain the strain was first noticed by Theobald (1912), since when it has briefly been dealt with by Edwards (1935), Triffitt (1931), Walton *et al.* (1933), as well as being reported from numerous widely distributed areas in this country (see *Reports on Pests of Crops* issued by the Ministry of Agriculture, 1928–37).

Although the strains of *Heterodera schachtii* are generally recognized to be invariably associated with the manifestation of a pathological condition of the host plants commonly known as "sickness", e.g. pea sickness, yet the majority of workers conducting investigations upon the bionomics of the nematode hold the opinion that this eelworm is only a contributory factor and that additional factors play an important part in the production of the typical syndrome of "sickness". Thus Morgan (1926), Smith & Miles (1929) and Miles (1933) concluded from their investigations upon the potato strain of *H. schachtii* that the eelworm alone is not sufficient to produce the pathological condition of the host plants known as potato sickness, while Carroll (1933) found that the intensity of this disease is directly proportional to the cyst content of the soil.

The biological races of this nematode parasitic upon sugar beet, potato and oats have been investigated by many workers both in this country and on the Continent but the pea race has been almost entirely neglected. In view of this fact and since this particular race has now become a menace to pea culture in many localities in this country, the present investigations were undertaken.

2. Symptoms and nature of pea sickness

The details of the pathological changes shown by diseased plants examined during the course of the experiments were found to vary with the intensity and duration of the sickness, but throughout its progress the symptoms are characteristic. Infected plants never make good growth and are unthrifty in appearance, even if they succeed in attaining maturity and in forming pods. They are much dwarfed and their stems are weak, thin and paler in colour than those of healthy plants.

The foliage is also characteristically affected, since it is pale green or yellow in colour and presents an abnormal appearance. The weak, crumpled laminae of the leaves are never as large and strong as those of healthy plants. With the final onset of the disease, all the leaves become limp and flaccid. The upper, younger leaves remain partially unopened and are closely set together, since the apical internodes fail to elongate

because of the retardation of growth of the plant. The lower leaves are curled and withered, eventually dropping off the stem. A gradation in the pathological manifestations of the disease in the foliage can be observed from the dead, brown basal leaves to the pale green undeveloped ones at the apex of the shoot. The foliage on the whole has a distinct yellow tinge, which becomes more apparent as the disease progresses and is conspicuous when a large number of sick plants are seen together, as in a field.

The flowers are small and never as large as those on healthy plants; they are not freely exposed on account of the exceedingly short internodes of the stem and the crowding together of the leaves at the apex of the shoot. In consequence, the bloom of affected plants is situated at the apices of the shoots which, in contrast to healthy ones, branch but slightly.

The root system of a badly attacked plant is poor and impoverished, and if examined at the opportune time will be found to be heavily infected with the cystic females of *H. schachtii*. The primary root is short, and very early in the life of the plant shows a considerable degree of decay towards its base. The secondaries, besides being short, are brown and soft in texture in contrast to those of a healthy plant which are long, firm and white in colour. The bacterial nodules, so plentiful and universal upon the roots of vigorous plants, are greatly reduced in number and may be entirely absent in severe cases.

In addition to these aerial and root symptoms, the stem at ground level may be partly or completely encircled by a dark brown ring of diseased tissue which was found in the present studies to occur only in the presence of the fungus *Aphanomyces euteiches* Drechsler.

3. Experiments on the determination of the factors involved in the causation of pea sickness

A quantity of soil that was definitely known to be sick, inasmuch as pea plants grown in it the previous year were badly diseased and had shown the various pathological changes characteristic of pea sickness, was obtained. As a preliminary experiment, several samples of this soil, after it had been well mixed, were potted and planted with peas. The young plants were carefully watered and kept under constant supervision throughout the period of growth. All of them in due course became sick, wilted and died, presenting the usual facies of pea sickness.

Examination of the plants revealed that *H. schachtii* was present in

the roots in all stages of development. Another nematode, *Rhabditis brevispina* Claus (1862), was also discovered infesting the plants, but in all cases this species appeared in the tissues of dying hosts only and was never found in actively growing plants. In addition, the fungus *Aphanomyces euteiches* Drechsler (1925) was discovered invading the plant tissues at the junction of the stem and root.

Although the probable causal factors were thus ascertained, it was obviously possible that, in addition, one or more unknown factors might be involved, without which typical pea sickness would not be manifested. The problem, therefore, resolved itself into an attempt to evaluate the importance of the rôles of *Heterodera schachtii* and *Aphanomyces euteiches* and to determine the presence or absence of one or more unknown factors, and, in the former event, to assign to the factor or factors their proper place in the causation of pea sickness. It was evident that the various agencies concerned would need to be studied individually and in all possible associations with one another, and that experiments to this end would entail the isolation and combinations of the factors discovered and the potential but unknown ones.

It was assumed that all the causal factors would be removed from the infected soil by a process of sterilization; certainly the nematode cysts and the fungal spores would be non-viable after such treatment. With the object of modifying the infected soil as little as possible, sterilization by steam rather than by chemicals was chosen. In view, however, of the stimulating action of steam sterilization upon plant growth, it was evident that untreated soil would need to be utilized as a control in order to estimate the beneficial effects on the plants resulting from such treatment.

The cysts of *Heterodera schachtii* on account of their relatively large size, having an average diameter of 0·5 mm., can be removed from infested soil by sieving, but the sieved soil would still be contaminated with the fungus spores. Apart from the removal of the cysts and larger soil particles, the composition of the sick or sieved soil would be maintained, since the unknown factors, unless they were inherent in the cysts or in the coarser components of the soil, would still be present. The texture of the soil would, however, be modified by the removal of the coarser material unless it was replaced by some substitute.

The removal of the fungal spores offered insuperable difficulties, but it was possible to culture the fungus for the purpose of inoculation, so that soil previously uninfected could be infected with it as desired.

Thus the factors *Heterodera schachtii* and *Aphanomyces euteiches*, as

well as the latter plus the unknown factors, if any, could be experimented with either singly or in association with each other, but the adoption of steam sterilization made the provision of "controls" essential. For these controls, soil known to be free from both *Heterodera schachtii* and *Aphanomyces euteiches* was used. Since both the eelworm and the fungus could be inoculated into this untreated, non-infected soil, the effects of the problematical and unknown factors could be estimated by their absence.

Culture of Aphanomyces euteiches *and the preparation of inoculum*

According to Drechsler (1925), as was later confirmed in the course of the present studies, the fungus *Aphanomyces euteiches* can only be induced to grow on artificial media by its transference during the short period when it is actively growing within the tissues of the host. For this reason young seedlings showing the first symptoms of fungal attack were selected. The basal portions of the stems were removed and thoroughly washed several times in sterile water before being placed on sterile plates of prune and pea agar.

A sparse growth was obtained on a few of these plates which quickly outgrew the bacterial colonies that developed around the various pieces of stem. Subcultures were then made by transferring some of the sterile portions of the plate, traversed by hyphae, to fresh plates of maize meal agar. These were then used as stock cultures since the abundant oospores produced by the fungus, when growing in this medium, will germinate readily upon this maize meal agar and continue to grow until the dish is covered by a densely compacted mass of aerial mycelia bearing enormous numbers of oöspores. It was decided to use the maize meal cultures at the height of the production of oospores for the purpose of making inoculations, since the oospores germinate very readily in water, and healthy seedlings placed in soil mixed with a relatively large amount of inoculum readily became infected by the parasite.

Collection of the cysts of Heterodera schachtii *and their preparation for inoculation*

The resistant stage of the eelworm is the durable cyst containing numerous eggs, each of which has a larva within it capable of emerging to infect a host plant, and, in order to transfer the nematode to soil formerly free from it, it was necessary to collect supplies of these cysts.

Two methods of collecting cysts had been in use by previous workers. The first, Carroll (1933), depends upon the collection of cysts from the

roots of infected plants, but this method could not be utilized in the present work. The second, Morgan (1925), is based upon the fact that the cysts will float on water. The technique consists in shaking small amounts of soil varying from 25 to 100 g. in a long-necked flask filled with water. The debris, together with the cysts, floats to the surface of the water and is then transferred to a filter paper from which the cysts are separately collected by hand. This method was improved upon in the present experiments by the use of an apparatus, which will be described in a subsequent paper, devised to extract cysts from several pounds of soil at one time.

The following method was adopted for the purpose of inoculating the various series. As the weight of soil required to fill each pot was 12 lb. this amount of pea sick soil was passed through the cyst collecting apparatus in three equal instalments. The supernatant matter, including the cysts, was sterilized by immersion in "10 volumes" hydrogen peroxide for a period of 30 min., when the surface-sterilized cysts were removed and washed in sterile water.

The cysts from each 12 lb. of affected soil were now uniformly distributed throughout the same weight of the soil to be inoculated, and the latter restored to its appropriate pot.

Triffit (1931) had employed hydrogen peroxide for the surface sterilization of *H. schachtii* cysts, but before using this technique the present writer ascertained the effects of the treatment upon the cysts and the contained larvae. Sterilized cysts were placed upon plates of nutrient agar, and since no bacterial or fungal growths developed, it was apparent that the peroxide had destroyed any extraneous spores on the surface of the cysts. Sterilized cysts were later dissected and the contained larvae found to be viable and active.

The sterilization of the surfaces of the cysts rendered the spores of *Aphanomyces euteiches* or any other organism innocuous so that only *Heterodera schachtii* would be inoculated into the soil.

Plan of pot experiments

Pot experiments seemed to offer the best method for the elucidation of the rôles played by the different factors, since each could be isolated and studied critically in relation to the plants under almost identical conditions.

Eleven series were set up. In each series, there were six 8½ in. earthenware flower pots, each holding 12 lb. of soil. All the pots had been steam sterilized and provided with adequate amounts of sterile material for drainage.

A quantity of pea-sick soil was obtained and air-dried until it could be easily pulverized. It was passed through a $\frac{1}{2}$ in. mesh sieve to remove the larger stones and thoroughly mixed until its constitution was uniform as regards its general appearance and cyst concentration. When similar cyst counts on samples of identical weight from different portions of the heap of soil were obtained, using a modification of the technique described by Morgan (1925), it was considered that homogeneity with regard to cyst distribution had been attained. The soil passing through a sieve of 2 mm. mesh was taken in each case in the examination for cysts.

The different series of pots were as follows:

Series I. Untreated pea-sick soil. Apart from the preparation already described the soil used in this series received no further treatment.

Series II. Steam-sterilized pea-sick soil. Soil similar to that in series I, except that it had been steamed in a Koch sterilizer for 2 hr. at the temperature of 98° C., was used.

Series III. Sieved pea-sick soil plus sand. The soil was identical with that used in series I except that it had been passed through a sieve of $\frac{1}{60}$ in. mesh with the object of removing the eelworm cysts. In order, so far as possible, to restore its original physical composition, the soil was thoroughly mixed with clean, dry, sterilized, coarse grained sand equal in weight to the discarded residue.

Series IV. Steam-sterilized pea-sick soil inoculated with *Aphanomyces euteiches*. The soil received a treatment similar to that in series II and, in addition, a standard amount of plated agar supporting a uniformly heavy growth of *A. euteiches* was intimately incorporated with it at the time of sowing.

Series V. Steam-sterilized pea-sick soil inoculated with cysts of *Heterodera schachtii*. The soil received a treatment similar to that in series II except that it was inoculated a few days before sowing with a number of cysts equivalent to that destroyed during the process of sterilization.

Series VI. Steam-sterilized pea-sick soil inoculated with both *Aphanomyces euteiches* and *Heterodera schachtii*. The soil received a treatment similar to that in series II except that it was inoculated with equivalent amounts of *Aphanomyces euteiches* and cysts of *Heterodera schachtii*, as described in series IV and V respectively.

Series VII. Untreated non-infected soil. The soil used in this series had been proved to be free from *Aphanomyces euteiches* and cysts of *Heterodera schachtii* by previously rearing uninfected plants in samples of it. In texture and composition it was highly similar to that selected for series I and received essentially the same preparation.

Series VIII. Non-infected soil infected with *Aphanomyces euteiches*. Soil similar to that used in series VII was inoculated with an amount of *A. euteiches* equivalent to that used in series IV and VI.

Series IX. Non-infected soil inoculated with cysts of *Heterodera schachtii*. Soil similar to that in series VII was inoculated with a number of cysts equivalent to that added in series V.

Series X. Non-infected soil inoculated with *Aphanomyces euteiches* and *Heterodera schachtii*. Soil similar to that in series VII inoculated with equivalent amounts of *Aphanomyces euteiches* and *Heterodera schachtii* as used in series VIII and IX, respectively.

Series XI. Sterilized non-infected soil. Soil similar to that in series VII was steam-sterilized as described for series II.

It was known that all the conditions necessary for the production of the disease

were present in the pea-sick soil, as used in series I, since the crop sown in the field from which it was obtained had been a total failure in the previous year on account of this malady.

The sterilization of the soil was carried out in April, and on 12 May 1936, twelve healthy surface-sterilized pea seeds kept in wet, sterile sand until they had commenced to germinate, were evenly planted at a depth of $1\frac{1}{2}$ in. in each pot. The plants of each series were subsequently watered whenever necessary, depending on the weather conditions, on each occasion equal volumes of water being applied to the different pots in all the series.

To ensure that ample and readily available supplies of all the necessary inorganic nutritive substances would not be lacking, an artificial fertilizer of the following composition and proportion was incorporated with the soil of each pot of every series immediately before planting:

Ammonium sulphate	19·70 g.	1 part
Potassium sulphate	39·40 g.	2 parts
Superphosphate	59·10 g.	3 parts

1·64 g. of the mixture was added to each pot of 12 lb. of soil, a dressing equivalent to 6 cwt. of fertilizer per acre of soil.

Effects of the treatments on plant growth and the manifestation of the disease

One week after planting, shoots of the seedlings had appeared above the surface of the soil in every pot. The first well marked indications of attack by *Aphanomyces euteiches*, namely the browning and weakening of the basal portions of the stem, were detected towards the end of the second week in June. The symptoms were well marked in the plants of series I, less noticeable in those of series III, and were not present in those of the artificially inoculated series, namely, Nos. IV, VI, VIII and X. The plants of these latter series were found, however, at a later date, to be infected, though never to the same degree as those in series I and III.

Towards the middle of June the effects of the various treatments were already manifesting themselves as regards the number of plants and the amount of foliage growth in the different series. The plants in series II, VII and XI showed a much more vigorous growth than those in the remaining series.

Those series which had been inoculated with *A. euteiches*, either in the absence (series IV, IX) or the presence (series VI and X) of *Heterodera schachtii*, as well as those series (I and III) which were naturally infected, included fewer plants than those not inoculated with this fungus (series V, IX).

By 18 August the plants had matured but the majority of the pods were still green. At this date the differences between the plants of the various series were more pronounced than at any time earlier in the season and brief notes on each series follow:

Series I. Untreated pea-sick soil. All the plants were definitely very sick, being stunted and with thin, weak stems varying from 1½ to 1¾ ft. in height. The leaves were small and unhealthy in colour varying from a sickly yellow near the base of the main stem to yellowish green towards its apex. The pods were small, few in number and invariably almost empty. In each pot one or more plants had died before reaching the miserable maturity attained by the remainder. In short, all the typical symptoms of pea sickness were exhibited by all the plants in this series, including the presence of the cysts of *H. schachtii* on the roots. In addition, the characteristic brown discoloration was shown at the base of the stem due to an attack by *Aphanomyces euteiches*.

Series II. Sterilized pea-sick soil. The plants were unusually vigorous and healthy with tall thick stems averaging from 3½ to 4 ft. in height, large, dark green healthy leaves and numerous well developed, full pods. Signs of disease were entirely lacking and although the plants were beginning to die down, none had perished during the season.

Series III. Sieved pea-sick soil. The plants were small and stunted, measuring from 1½ to 2 ft. high, their stems being very slender and bearing poorly developed pale green leaves. The pods were few in number and almost empty. The usual symptoms of parasitization by *A. euteiches* were present on nearly all the plants, several of which had already succumbed to the effects of this fungus.

Series IV. *A. euteiches* inoculated into sterilized pea-sick soil. The plants were healthier in appearance than those in series I and III. They measured 2½ ft. or more in height and possessed pale green foliage.

Series V. *Heterodera schachtii* inoculated into sterilized pea-sick soil. The plants were obviously diseased, the foliage being very pale and the yield of crop exceedingly small. The heights of the plants averaged about 2 ft.

Series VI. *Aphanomyces euteiches* and *Heterodera schachtii* inoculated into sterilized pea-sick soil. The plants were even poorer in appearance than those in series IV and V, indicating that the combined effects of both parasites were greater than those due to either alone.

Series VII. Untreated non-sick soil. The plants evidenced no pathological symptoms of any kind and measured on an average 3–3½ ft. in height. The stems were well developed and the leaves normal in colour. Some individual plants were still flowering though the greater number of them carried well filled pods. The plants, though exhibiting a good healthy growth, were inferior in appearance to those of series II and XI.

Series VIII. Non-sick soil inoculated with *Aphanomyces euteiches*. The plants were on the whole fewer in number and not so flourishing as those in series VII. The foliage was of a dull green colour and the pod production rather poor. Symptoms of fungal attack were not, however, very evident.

Series IX. *Heterodera schachtii* inoculated into non-sick soil. The plants, though somewhat variable in appearance in the different pots, were definitely retarded in

comparison with those of series VII. They measured only about 2½ ft. in height and showed all the typical symptoms of pea sickness with the exception of the dark discoloration at the base of the stem, which is associated with *Aphanomyces euteiches*.

Series X. *Aphanomyces euteiches* and *Heterodera schachtii* inoculated into non-sick soil. This series included plants which were inferior to those of series VII, VIII and IX. The whole symptoms of disease were not, however, generally so well marked as in series VI.

Series XI. Sterilized non-sick soil. The plants were definitely superior to those in series VII and comparable in all respects with series II. The general appearance, as in series II, gave the impression of great robustness and unusual vigour both of the vegetative portions and of the pods, thus demonstrating the beneficial effects of the process of steam sterilization of the soil.

Pls. XXXIII and XXXIV, which were taken in July, illustrate the effects of the various treatments upon the plants of the different series. It will be noted (fig. 1) that the symptoms of pea sickness were very marked in the plants in the pot from series I, particularly the dwarfed, thin stems and the poor pale coloured foliage, and the luxuriant growth and fine development of the plants of series II is in marked contrast. It is evident, therefore, that the pathogenic factors were removed by the process of steam sterilization and that the plants were more than usually robust. The pot from series III shows diseased plants which were superior to those of series I, but much inferior to those in series II. It may be mentioned in this connexion that the replacement of the coarse gravel removed from the soil by sieving was not adequately compensated for by the addition of an equal weight of sand. In contrast to the soil in the other ten series, the mixture of sand and sieved soil in this series resulted in the formation of a tenacious, close grained compost which was almost impermeable to water, thus depriving the plants growing in it of an abundant supply of moisture and air for their normal development. The plants of this series, as already stated, were badly infected by *Aphanomyces euteiches* which was probably aided in its parasitization by the tenacious character of the soil.

The plants of series IV, V and VI, which are shown in fig. 2, illustrate the adverse effects of the fungus and the eelworm, acting separately or in conjunction with one another. It is apparent that the combined effects of both parasites were cumulative and that the plants of all three series were considerably inferior to those of series II (fig. 1).

Similarly, fig. 3, showing a specimen pot of each of series VIII, IX, and X also illustrates a sequence of the cumulative effects of these two parasites. It is evident that the plants in all these pots are considerably less vigorous than those of the untreated series (VII), which is shown in

fig. 4; the adverse influences of the fungus and the nematode were again very marked and of the two organisms, the latter proved to be the more important pathogenic agent. The sickness is more pronounced in the inoculated unsterilized series (VIII, IX and X), than in the corresponding inoculated sterilized series (IV, V and VI). This difference is attributable to the stimulative effect which the process of sterilization has upon plant growth.

Fig. 4 shows typical pots from series IX, VII and XI and demonstrates the effect of *Heterodera schachtii* alone. In contrast to the plants of series VII and XI, those of series IX are obviously sick. There can be no doubt, therefore, of the significance of the nematode as the causative agent of pea sickness, while the effect of steam sterilization in stimulating plant growth to a marked degree is well shown.

4. Conclusions

The results of the experiments described show that in the pea-sick soil studied, the eelworm *Heterodera schachtii* and the fungus *Aphanomyces euteiches* were both concerned in the production of a diseased condition of pea plants in the absence of any other factor of an organic nature. Either of these organisms can produce pathological symptoms in the absence of the other, and the effect upon the plants of infection by *Heterodera schachtii* alone indicates that it is the primary factor causing pea sickness.

5. Summary

1. A series of experiments was carried out upon the etiology of pea sickness, a disease which is invariably associated with *Heterodera schachtii*.

2. In addition to this eelworm, a fungus, *Aphanomyces euteiches*, was found to be concerned in the production of the diseased condition of the plants in the soil studied.

3. Both *Heterodera schachtii* and *Aphanomyces euteiches* can singly produce a pathological condition of the host in the absence of any other pathogenic factor.

4. The effects upon the plants of attack by *Heterodera schachtii* alone indicate that it is the primary cause of pea sickness.

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REFERENCES

Capus, J. (1917). Sur le déperissement des cultures de pois en Gironde de la double action de l'*Heterodera schachtii* et du *Fusarium vasinfectum*. *Bull. Soc. Zool. Agric. Bordeaux*, **16**, 70, 87.

—— (1918). Invasion des cultures de pois en Gironde par *Heterodera schachtii* Schmidt. *Ann. Epiphyt.* **5**, 239.

Carroll, J. (1933). A study of the potato worm, *Heterodera schachtii* in the Irish Free State. *J. Helminth.* **11**, 137.

Claus, C. (1862). Ueber einige in Humus lebende Anguillulinen. *Z. wiss. Zool.* **12**, 354.

Cornu, K. (1879). Études sur le *Phylloxera vastratrix*. *Mém. prés. Acad. Sci., Paris*, **26**, 1–357.

Drechsler (1925). Root rot of peas in the United States caused by *Aphanomyces euteiches* (n.sp.). *J. agric. Res.* **30**, 293.

Edwards, E. E. (1935). On *Heterodera schachtii*, with special reference to the oat race in Britain. *J. Helminth.* **13**, 129.

Goodey, T. (1932). On the nomenclature of the root-gall nematodes. *J. Helminth.* **10**, 21.

Kühn, J. (1874). Ueber das Vorkommen von Rübennematoden an den Wurzeln der Halmfrüchte. *Z. Ver. Rübenzucker Ind. Zollver.* **24**.

Liebscher, C. (1890). Eine Nematode als Ursache der Erbenmüdigkeit des Bodens. *Dtsch. landw. Pr.* **17**, 436, 672.

Miles, H. W. (1933). Field studies on *H. schachtii* Schmidt, in relation to the pathological condition known as "Potato sickness". *J. Helminth.* **8**, 103.

Morgan, D. O. (1925). Investigations on eelworm in potatoes in south Lincolnshire. *J. Helminth.* **3**, 185.

—— (1926). Some remarks on the etiology of potato sickness in Lincs. *J. Helminth.* **4**, 49.

Schacht, H. (1859). Ueber einige Feinde und Krankheiten der Zuckerube. *Z. Ver. Rübenzucker Ind. Zollver.* **9**, 390.

Schmidt, A. (1871). Ueber den Rüben-Nematoden (*Heterodera schachtii* A.S.). *Z. Ver. Rübenzucker Ind. Zollver.* (V. Sch), **21**, 1.

Smith, A. M. & Miles, H. W. (1929). Investigations on *Heterodera schachtii* in Lancashire and Cheshire. Pt. III. *Ann. appl. Biol.* **16**, 596.

Theobald, F. V. (1912). Report on economic zoology for year ending 30 September 1912. *J. S.-E. Agric. Coll.* no. 21, p. 173.

Triffitt, M. J. (1931*a*). On the pathogenicity of *Heterodera schachtii* to potatoes and mangolds. *J. Helminth.* **9**, 1.

—— (1931*b*). On the eelworm *Heterodera schachtii* attacking peas in Britain. *J. Helminth.* **9**. 175.

Walton, C. L., Ogilvie, L. & Mulligan, B. O. (1933). Observations on the pea strain of the eelworm *Heterodera schachtii* and its relation to "pea-sickness". *Rep. agric. hort. Res. Sta. Bristol*, p. 74.

EXPLANATION OF PLATES XXXIII AND XXXIV

PLATE XXXIII

Fig. 1. Series I. Pea plants grown in "pea-sick" soil. Series II. Pea plants grown in sterilized "pea-sick" soil. Series III. Pea plants grown in sieved "pea-sick" soil.

Fig. 2. Series IV. Pea plants grown in "pea-sick" soil, sterilized and reinfected with *Aphanomyces euteiches*. Series V. Pea plants grown in "pea-sick" soil, sterilized and reinfected with *Heterodera schachtii*. Series VI. Pea plants grown in "pea-sick" soil, sterilized and reinfected with both *Aphanomyces euteiches* and *Heterodera schachtii*.

PLATE XXXIV

Fig. 3. Series VIII. Pea plants grown in eelworm and fungus-free soil infected with *Aphanomyces euteiches*. Series IX. Pea plants grown in eelworm and fungus-free soil infected with *Heterodera schachtii*. Series X. Pea plants grown in eelworm and fungus-free soil infected with both *Aphanomyces euteiches* and *Heterodera schachtii*.

Fig. 4. Series IX. Pea plants grown in eelworm and fungus-free soil infected with *Heterodera schachtii*. Series VII. Pea plants grown in eelworm and fungus-free soil. Series XI. Pea plants grown in sterilized eelworm and fungus-free soil.

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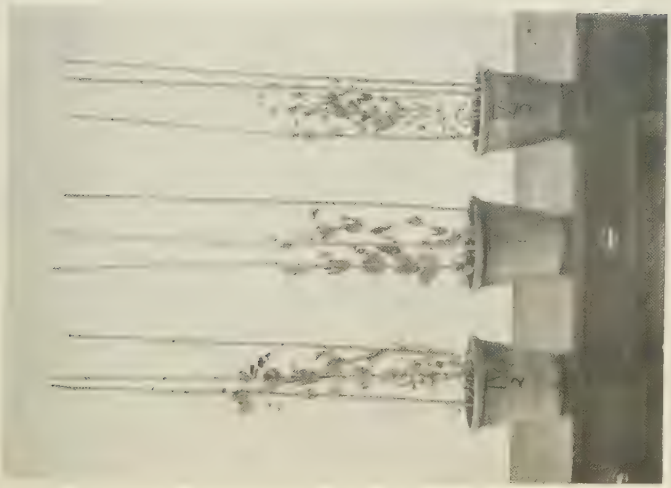

Fig. 2.

Fig. 1.

POWELL.—Investigations upon the Pea Strain of *Heterodera schachtii* Schmidt and its Rôle in the Causation of Pea Sickness (pp. 572–584)

Fig. 4.

Fig. 3.

POWELL.—Investigations upon the Pea Strain of *Heterodera schachtii* Schmidt and its Rôle in the Causation of Pea Sickness (pp. 572–584)

THE TOXICITY OF POISONS APPLIED JOINTLY[1]

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(With 14 Text-figures)

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Introduction

With the development of quantitative methods for the estimation of toxicity, a study of the mode of action of poisons applied jointly has acquired new potentialities. Some of this interest is theoretical and concerns the statistical approach to the relation between dosage and percentage kill. The typical curve relating these two variables is asymmetrical and sigmoid, but it can be transformed to a straight line and computed with measurable precision by methods first introduced by Gaddum (1933). Empirical tests have shown the wide applicability of the technique to a large number of animal species and poisons. Cases have been reported, however, in which the slope of the rectified dosage-mortality curve has changed more or less abruptly, usually at the lower dosages. When the population of test animals was reasonably uniform, the most general explanation of these "breaks" has been that each segment of the rectified curve represented a specific type of toxic action, such as might be produced by one constituent in a complex poison. It is desirable to show, therefore, what would result with different mixtures that are assumed or known to contain two toxic ingredients.

The subject is also of practical interest. In the search for new insecticides combined poisons offer many possibilities, but criteria are

[1] This paper is an extension of a report on synergism written by the author in June 1937, at the Laboratory of Insect Toxicology of the Institute for Plant Protection, Leningrad, U.S.S.R.

needed for separating mixtures in which the combined ingredients possess an enhanced toxicity from others in which they act independently since the former group provides the more promising field of investigation. Those investigating these possibilities have not yet agreed either upon experimental procedure or upon the meaning of their results. In a study on the toxicity of rotenone-pyrethrin sprays, for example, Le Pelley & Sullivan (1936) concluded that synergistic action was absent. Yet, in a footnote based upon the same original data, H. H. Richardson found definite evidence of synergism. To resolve uncertainties of this nature the quantitative problems underlying the toxicity of combined poisons and drugs should be examined in more general terms than heretofore. With this in view the present paper is concerned primarily with the definition and quantitative analysis of three types of joint toxic action in which the percentage mortality is used as the measure of response.

Types of joint action

The three types of joint action can be identified from the relative toxicities of the separate constituents when applied singly and in mixtures. The most general measure of toxicity and the only one considered here is the percentage kill and the dosage-mortality curve based upon it. The computation of this curve has recently been summarized by Bliss (1938). As the simplest case we will assume that the characteristic curve for each constituent in a given mixture can be plotted as a single straight line when the dosage is transformed to logarithms and the observed percentage mortality to probits or their equivalent. The equation of the curve can then be written in the simple form

$$Y = a' + b \log D, \qquad \text{.....(1)}$$

where Y is the expected mortality in probits at any given dosage D, and a' and b are constants computed from the experimental data. The regression coefficient b measures the rate of change in mortality per unit change in the log dose, and its reciprocal, $1/b$, is the standard deviation of sensitivity (or resistance) to the drug or poison. The degree of toxicity, which is determined by a', is independent of the slope or standard deviation, but it is usually more convenient to measure toxicity in terms of the median lethal dose (LD50) or its logarithm rather than in terms of a'. To obtain log LD50 from equation (1), Y is set equal to 5 and the equation solved for log D. As the mid-point of the complete curve it is the standard measure of toxicity.

When two poisons are combined or administered jointly, we may

specify further that the toxicity of the mixture must be determined at several dosages. This is now standard procedure in many cases. It has a twofold objective: (1) to determine whether the dosage mortality curve for the mixture is linear over the full range of mortalities, a point of diagnostic value, and (2) to compute the amount of mixture required to kill fifty or some other specified percentage of the test animals. Usually the ratio of the constituents can be kept constant by preparing a standard solution which is then diluted to obtain the required series of doses. It is essential, of course, that the components be compatible chemically under the conditions of the experiment. If they react to form a stable compound prior to application, this is a complication beyond the present scheme, for study by a chemical technique. The classification below is based entirely upon relative toxicities.

With these limitations, we may recognize three principal types of joint action:

(1) *Independent joint action.* The poisons or drugs act independently and have different modes of toxic action. The susceptibility to one component may or may not be correlated with the susceptibility to the other. The toxicity of the mixture can be predicted from the dosage-mortality curve for each constituent applied alone and the correlation in susceptibility to the two poisons; the observed toxicity can be computed on this basis whatever the relative proportions of the components.

(2) *Similar joint action.* The poisons or drugs produce similar but independent effects, so that one component can be substituted at a constant proportion for the other; variations in individual susceptibility to the two components are completely correlated or parallel. The toxicity of a mixture is predictable directly from that of the constituents if their relative proportions are known.

(3) *Synergistic action.* The effectiveness of the mixture cannot be assessed from that of the individual ingredients but depends upon a knowledge of their combined toxicity when used in different proportions. One component synergizes or antagonizes the other.

These three types of joint action are sufficiently well defined that they can be distinguished with little difficulty, although there probably are other cases which fall between or outside of these classifications.

Independent joint action

The simplest case is that in which the two components act independently of each other. Presumably each involves a characteristic and distinct chain of reactions in the organism, so that the animal dies from

the failure of a different vital system in the one case than in the other. Susceptibility to the one ingredient, however, may still be correlated to a greater or lesser degree with susceptibility to the other. Hough (1934) has shown, for example, that a race of codling moth which was more susceptible to ingested lead arsenate was also more susceptible to poisoning by hydrocyanic acid gas. Any estimate of the mortality expected from two poisons which act independently must take this factor into account.

The combined effect of two poisons, A and B, which act independently can be examined most readily in terms of the proportion killed. If there were no correlation in susceptibility to the two components, so that the coefficient of association, r, is equal to zero, then

$$p_C = p_A + p_B - p_A p_B, \qquad \ldots\ldots(2)$$

where p_C is the proportion of animals killed by the combination when p_A and p_B are the proportions dying from the same amounts of A and B applied alone. Entomologists will recognize this as equivalent to the so-called "Abbott's formula" for computing from the observed total mortality the effectiveness of a poison, p_B, in the presence of a given natural mortality, p_A. For this purpose it is usually written as

$$p_B = \frac{p_C - p_A}{1 - p_A}. \qquad \ldots\ldots(2a)$$

If, on the other hand, there should be an exact parallelism in the susceptibility to the two poisons, so that $r = 1$, then any individual receiving a fatal dose of the less toxic ingredient would receive a more than fatal dose of the more toxic ingredient,

$$p_C = p_A, \qquad \ldots\ldots(3)$$

where $p_A > p_B$, and the combined mortality would be the same as that attributable to the more toxic ingredient applied alone. Any more general equation which will cover intermediate degrees of association as well must reduce to equation (2) when $r = 0$ and to equation (3) when $r = 1$. These requirements are met most simply by

$$p_C = p_A + p_B\,(1 - p_A)\,(1 - r), \qquad \ldots\ldots(4)$$

where $p_A > p_B$. Any case in which the mortality due to a combination poison can be estimated by equation (4) from that of the two ingredients applied separately, regardless of the percentage composition or dosage of the mixture, can be classified in this category of independent joint action.

The association between susceptibilities, r, can be computed readily from experimental data. Three dosage-mortality curves are required, one

for each of the two poisons applied alone and a third for the two applied jointly. A convenient mixture for this purpose is one in which A bears the same ratio to B as the LD50 of A to the LD50 of B, although mixtures containing other fixed proportions of the two ingredients can be used as well. The LD50 is computed from the dosage-mortality curve for each ingredient applied alone and the logarithm of their sum inserted in the equation of the curve for the combination to compute the expected kill in probits. This is then transformed to the proportion dead, p_C, by reference to a table of probits and the association of susceptibilities, r, computed from equation (4) as $r=3-4\,p_C$. Within the limits of experimental error it should be possible to compute the mortality observed with any combination from the dosage-mortality curves for the separate ingredients and a single coefficient of association. Combinations which fail to meet this requirement belong to another category of joint action.

Dosage-mortality curves for hypothetical mixtures. It is assumed in part of the above procedure that the percentage mortalities, obtained with different dosages of a toxic mixture, can be plotted and computed in terms of straight lines after transformation of the observed percentages to probits and of the combined dosages to logarithms. Since departures from linearity might prove of diagnostic value, the limitations to this assumption are of interest. It can be tested conveniently by plotting the expected mortality in probits against the log dose for mixtures of two hypothetical poisons. For this purpose the association in the susceptibility to the two poisons has been assumed to be 0·6, a value intermediate between complete association and its entire absence. Combinations of poisons involve several distinct possibilities.

The first point concerns the effect of different proportions of two ingredients upon the toxicity of the mixture. In order to emphasize the contrast between them, the LD50 of the two poisons has been selected at 10 and at 2 mg. for A and B respectively, with a fourfold difference in slope. The dosage-mortality curves for the ingredients were $Y_A=2{\cdot}5+2{\cdot}5X_A$ and $Y_B=1{\cdot}99+10X_B$, where Y is the mortality in probits and X the dose in logarithms. Four mixtures have been postulated, containing the following proportions of the two ingredients in terms of their LD50's: (1) $\frac{1}{5}$ of A to $\frac{4}{5}$ of B, (2) $\frac{2}{5}$ of A to $\frac{3}{5}$ of B, (3) $\frac{3}{5}$ of A to $\frac{2}{5}$ of B and (4) $\frac{4}{5}$ of A to $\frac{1}{5}$ of B. The mortality attributed to each component if given alone was then computed for various dosages of the mixtures from the above equations and transformed from probits to p_A and p_B, which were substituted in turn in equation (4) with $r=0{\cdot}6$ to find p_C, the combined mortality when applied jointly. After conversion

to probits the curves for the four mixtures have been plotted in Fig. 1 over a wider range of toxicities than would be determined in most experiments.

The most striking characteristic of Fig. 1 is that the dosage-mortality relation was not a smooth curve but fell into two segments which would be indistinguishable from straight lines if determined from actual biological data. Except for the mixture containing the largest proportion of the less active ingredient, the transition or "break" from one segment to the other was relatively abrupt. In other examples, not given

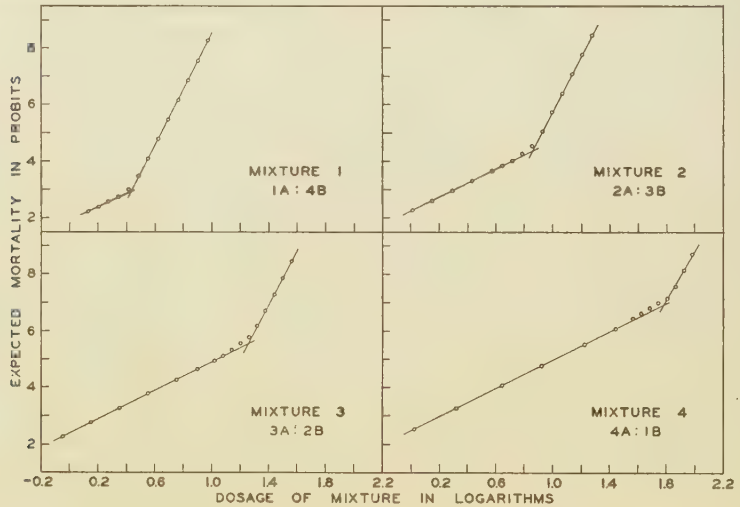

Fig. 1. The toxicity of four hypothetical mixtures containing specified proportions in equitoxic units of ingredients *A* and *B* on the assumption of independent joint action.

here, this break became more abrupt with larger values of the association coefficient, r, until with complete association between susceptibilities the slope changed at a sharp angle with no trace of curvature. Complete absence of correlation, on the other hand, increased the curvature at the break very little over that shown in Fig. 1. When the components differ in slope and act independently, therefore, the dosage-mortality curve obtained with a combined poison should contain clear evidence of its compound nature.

The extent to which the slope of each segment paralleled that of the constituent with which it is identified has been tested by computing (without weights) the straight lines drawn through each apparently linear series of plotted points. The flatter section in each case was

virtually uninfluenced by the other component, so that the original slope of $b = 2{\cdot}5$ recurred unchanged. Similarly, the slope of the steeper segment departed very little from the value for constituent B ($b = 10$) unless the break occurred at a relatively high mortality, the regression coefficients for mixtures 1–4 being 9·97, 9·97, 9·56 and 8·75 respectively. When the break occurred at less than 5 probits, the slope of the upper segment also isolated a single phase of toxic action from the complex.

The mixtures tested in Fig. 1 were based upon a single pair of hypothetical poisons. In order to test these conclusions with mixtures in-

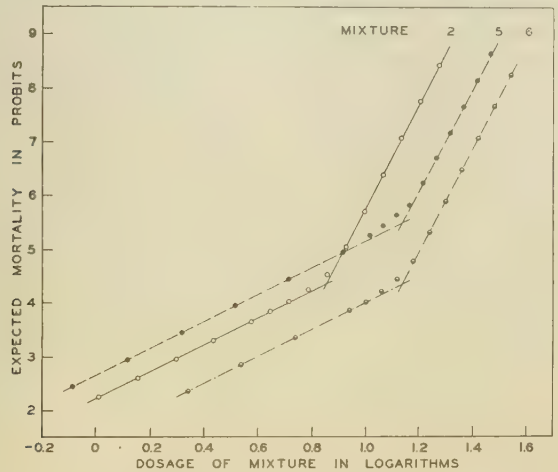

Fig. 2. The toxicity on the assumption of independent joint action of three hypothetical mixtures in which the LD50 of the ingredient with the steeper slope ($b = 10$) relative to that with the lesser slope ($b = 2{\cdot}5$) was taken to be greater (mixture 2), smaller (mixture 5) and the same (mixture 6). By definition there were 2 equitoxic units of the less toxic ingredient to 3 of the other.

volving other relations between the ingredients, three other combinations have been computed and plotted in Figs. 2 and 3. In each instance the mixture was assumed to contain 2 parts of A to 3 parts of B in terms of the LD50, quite as in the second mixture of Fig. 1 (repeated in Fig. 2 for comparison). The LD50's of the two poisons in mixture 5 were assumed to be the same as in mixture 2, but their slopes were interchanged so that the less toxic ingredient had the steeper slope, the dosage-mortality curves then being $Y_A = -5 + 10X_A$ and $Y_B = 4{\cdot}2475 + 2{\cdot}5X_B$. Although both mixtures 2 and 5 had nearly the same median lethal dose and the same general form, mixture 5 was more toxic below LD50 and mixture

2 above. In mixture 6 both ingredients were assumed to have the same LD50 (10 mg.), but the slope of one ingredient was taken to be four times that of the other. There was the same break in the dosage-mortality curve for the combination as when the components differed in toxicity and the LD50 for the mixture was larger than that for either alone. Finally, in mixture 7 (Fig. 3, Type I) both components were assumed to have the same slope ($b=5$) but to differ fivefold in toxicity. In this case the break observed with all other mixtures disappeared and the slope of the combination ($b_C=5{\cdot}150$) approached that of the original ingredients.

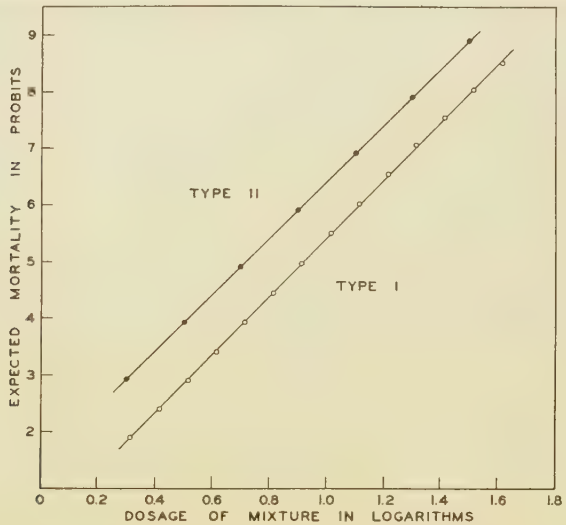

Fig. 3. The toxicity of a hypothetical mixture containing 2 equitoxic units of A to 3 of B where the LD 50 of B was one-fifth that of A but both had the same slope. They were assumed to act independently as postulated for Type I (equation (4)) in the lower curve and for Type II (equation (5)) in the upper curve.

It is evident from the above models that the dosage-mortality curve for any mixture in which the ingredients act independently will be discontinuous unless the curves for the individual components are parallel. It is also clear that recognizable characteristics of the original ingredients can be recovered quantitatively from the toxicological relations of the complex.

Experimental examples and discussion. Dosage-mortality curves resembling the models in Figs. 1 and 2 occur not infrequently in toxicological research. A convenient example has been given in a recent paper by Murray (1938) on the toxicity of a pyrethrum-kerosene spray to

female house-flies. The curve in Fig. 4 has been computed from his totals, which averaged 440 flies per concentration, and the section for the lower five concentrations has been determined separately from the upper six by the standard procedure. Pyrethrum extract is a mixture containing two principal ingredients which occur naturally together, known as pyrethrin I and pyrethrin II, pyrethrin I being much more toxic to house-flies than pyrethrin II. If we assume that Fig. 4 is a true picture of the dosage-mortality relations for a homogeneous population of female flies with this sample of pyrethrum, for which the relative proportions of pyrethrin I and II were not given, possibly the simplest explanation

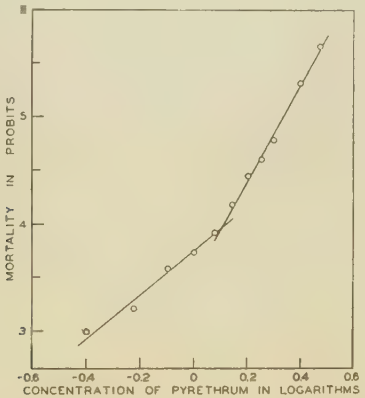

Fig. 4. The toxicity in a Peet-Grady chamber of atomized pyrethrum spray to female house-flies. Data of Murray (1938).

of the change in slope is that the lower flatter segment, for which $Y = 3{\cdot}628 + 2{\cdot}065(X + 0{\cdot}0575)$, has been determined principally by pyrethrin II and the steeper segment, for which $Y = 4{\cdot}836 + 4{\cdot}530$ $(X - 0{\cdot}2981)$, by pyrethrin I. On this hypothesis the two components acted independently, but from the relative abruptness of the change in slope individuals that were more susceptible to one ingredient were probably also more susceptible to the other in more or less the same order. Since this pyrethrum mixture was $3{\cdot}24 \pm 0{\cdot}03$ times as toxic to males as to females at LD50, the smallest concentration killed 23 % of the male flies, which was well above the level of mortality at which the change in slope occurred for the more resistant females. It was to be expected, therefore, that the male curve (not given here) would have a single slope over the present range of dosages, as was observed. The

hypothesis that the two segments of the female curve represent the two forms of pyrethrin is, of course, only one of several possibilities. Its correctness could be tested by determining under strictly comparable conditions the dosage-mortality curve for female flies with mixtures containing different proportions of pyrethrin I and II and especially with pure solutions of each ingredient if the latter are obtainable. Then the larger the proportion of pyrethrin II, the higher should be the probit mortality at which the change in slope occurs.

Of more general significance is the resemblance of the models shown in Figs. 1 and 2 to occasional discontinuous curves obtained experimentally with a single toxic agent. Bliss (1935) has suggested that if the amount of poison fixed by the organism were limited by adsorption, a change in the slope of the dosage-mortality curve might be explained in terms of the Langmuir adsorption theorem. Breaks of this sort occur, however, with many toxic agents where adsorption is not a factor, such as with lethal temperatures and X-rays. A more inclusive explanation of discontinuities in the dosage-mortality curve follows directly from Figs. 1 and 2.

Let us assume that a given toxic agent acts destructively and more or less independently upon two or more physiological systems in the animal. If these effects are quite distinct, it is not unlikely that they will differ also in their standard deviations (the reciprocal of slope). Then the dosage-mortality curve for a single poison could show a change in slope quite equivalent to that obtained by a mixture of two independent poisons, each with a single dominant effect. This explanation, of course, is that commonly known as the principle of the master reaction with the proviso that the limiting reactions exposed by the dosage-mortality curve must be distinct processes and not interdependent steps in a single continuous series. If these separate phases were merely links in a single chain, one would not expect the result to be independent of the relative proportions of the two ingredients as is postulated in the model.

This interpretation finds a useful extension to time-mortality relations such as are encountered in cage tests with insecticides, where insects are confined on poisoned food and the length of survival timed for each individual. Under such conditions it is common experience for the logarithm of the survival time to follow the normal distribution, so that it can be plotted as a straight line when the accumulated percentage mortality is transformed to probits and the elapsed time to logarithms, as has been described elsewhere (Bliss, 1937). The individuals in the control cages on standard food, however, usually die off at quite a different rate, so that in terms of probits and logarithms the curve is smoothly concave. A typical

series of each type has been taken from the cage tests by Boyce (1931) on the walnut-husk fly and plotted in Fig. 5. The straight line (*A*) shows the course of mortality on a sweetened fluosilicate dust and the curve (*B*) that on powdered sugar. The flies on the poisoned dust died from a single predominant toxic effect, those in the control cage from the multiplicity of causes involved in "natural mortality". Since these latter are probably diverse and presumably independent of each other, a curve such as Fig. 5 *B* is precisely what one would expect if the two toxic agents acting jointly in Figs. 1 and 2 were increased to five or ten or more which differ in their slope or standard deviation.

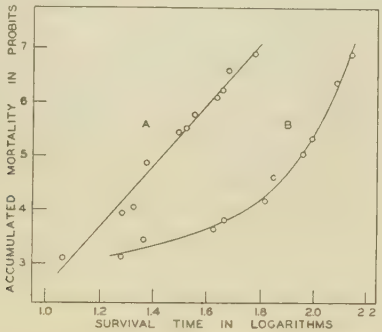

Fig. 5. Time-mortality curves for thirty-six walnut-husk flies confined with a sweetened dust containing 40 % of barium fluosilicate (*A*) and for thirty-five flies in a similar cage with powdered sugar (*B*). Data of Boyce (1931).

Similar joint action

The second type of joint toxic action is that in which two poisons produce similar but independent effects, so that in a mixture one can be substituted at a constant ratio for any proportion of the other without altering the toxicity of the combination. Since both ingredients act upon the same system of receptors within the animal, the dosage-mortality curves for both components and for any mixtures of the two should be parallel although they may differ materially as to median lethal dose. The curve for the mixture should show only such discontinuities or breaks as occur when the ingredients are tested alone. It follows from the criterion of independence that the toxicity of any combination, regardless of its percentage composition, can be predicted from the dosage-mortality curves of the ingredients. Presumably, the sensitivity to one poison is completely correlated with the sensitivity to the other, so that no

coefficient of association need be determined. Toxic mixtures of this type have a greater expected potency than those in the preceding class.

The quantitative relations of the combined poisons follow relatively simple forms. The dosage-mortality curve for a mixture of A and B is the same as that for A alone when computed in the form

$$Y_C = a' + b \log (D_A + kD_B), \qquad \text{.....(5)}$$

where D_A and D_B are the doses of A and B in the mixture and k is the ratio of toxicities of the separate ingredients. The ratio k is equal numerically to the anti-logarithm of a more general term, the log ratio of potencies, M. It is computed from the means of the dosage-mortality curves for the two ingredients as

$$M = \bar{x}_A - \bar{x}_B + \frac{\bar{y}_B - \bar{y}_A}{b_C}, \qquad \text{.....(6)}$$

where $\bar{x}_A$ and $\bar{x}_B$ are the mean log dosages and $\bar{y}_A$ and $\bar{y}_B$ the mean probits for the components A and B and b_C is the combined slope of the two curves. This equation is equivalent to equation (29) in Bliss (1935), to which the reader is referred for the standard error of M, s_M.

The dosage D_C, of a given mixture which will have a specified toxicity, can be estimated from the dose of ingredient A producing the same mortality, D_A, by a simple transformation of equation (5) to

$$D_C = \frac{D_A}{Q + k(1-Q)}, \qquad \text{.....(7)}$$

where Q is the proportion of A in the mixture. If the poisons can be applied only in combinations of known percentage composition which can be varied, k can be computed from the ratio of potencies for two different mixtures by means of equation (7). The value so obtained can then be applied to data from still other mixtures to test its consistency with the second type of joint action.

The greater toxicity of a combination drug or poison belonging to this as compared with the preceding class is illustrated by the two dosage-mortality curves in Fig. 3. The dosage-mortality equations for the separate components were assumed to be $Y_A = 0 + 5X_A$ and $Y_B = 3{\cdot}495 + 5X_B$ with $k = 5$. Two dosage-mortality curves have been computed for a mixture containing 2 parts of A to 3 parts of B in terms of the LD 50 or $Q = 0{\cdot}7692$. When the two ingredients were assumed to kill in diverse ways as determined by equation (4) with $r = 0{\cdot}6$, $Y_C = 0{\cdot}258 + 5{\cdot}150X_C$; under similar joint action as determined by equation (7), $Y_C = 1{\cdot}42 + 5X_C$. Some 38 % less of the combined poison would be needed to produce

equal mortalities in this case if the mixture followed the second rather than the first type of joint action.

Numerical example. As a numerical example we may examine the toxicity to adult house-flies of alcoholic solutions of rotenone and a single sample of pyrethrum, applied alone and in combination as reported by Le Pelley & Sullivan (1936). The mean probit mortality for 8–10 determinations with 100 flies each has been adopted as the measure of response to each of the five concentrations in every dosage-mortality curve. When plotted against the log concentration of the active principles in a given quantity of solution, the dosage-mortality curves for rotenone, pyrethrin and combinations of the two were parallel straight lines within the experimental error. Although the two poisons are known to have qualitatively different effects, the parallelism of their dosage-mortality curves and the conclusion reached by the experimenters suggested that mixtures of the two might agree with the second type of joint action. Since all of the combined mortalities exceeded 4 probits and thus fell within the range where, from a preceding example, the effect of pyrethrin I would be expected to dominate that of pyrethrin II, the rotenone-pyrethrin mixtures could be tested as if they were two-poison systems of pyrethrin I and rotenone.

The experiment consisted of two series of tests. Since the flies in the second series showed a somewhat greater susceptibility than those in the first, direct comparisons were limited to curves in the same series. Both ingredients were tested alone in each series. Since their relative toxicities agreed, the two estimates of M have been combined to give $M = 0{\cdot}780 \pm 0{\cdot}018$ or $k = 6{\cdot}03$. By multiplying all concentrations of rotenone in mg. per c.c. by 6·03, the data on rotenone could be superimposed over that for pyrethrum in each series as shown in Fig. 6. A dosage-mortality curve has been computed for each series on a pyrethrum level, that for series I being $Y = 5{\cdot}193 + 3{\cdot}844\ (X - 1{\cdot}016)$ and that for series II, $Y = 5{\cdot}249 + 3{\cdot}498\ (X - 1{\cdot}011)$. If the joint potency of rotenone-pyrethrin solutions conformed to the second type, they should agree with these equations and the solid lines in Fig. 6 when converted to a pyrethrum basis.

Two different proportions of rotenone and pyrethrum were tested, a mixture in which the amounts of each component were of approximately equal potency, represented by the five concentrations in series I, and a second mixture containing a little less than 4 equi-toxic parts of pyrethrin to 1 of rotenone, represented by the five concentrations in series II. The observed kill in probits at each concentration in the two series has been plotted against log $(D_A + 6{\cdot}03 D_B)$, where D_A was the concentration of

pyrethrin and D_B of rotenone in mg. per c.c., as shown by the black circles and broken lines in Fig. 6. The mixture in series II had essentially the same toxicity as the components applied alone, but that in series I was consistently more toxic than the two constituents. The significance of the difference has been tested by computing from the dosage-mortality curves for the mixture and its components the log ratio of potencies, M, and its standard error, s_M. For series I, $M = -0{\cdot}0496 \pm 0{\cdot}0208$ with $t = 2{\cdot}380$ and $n = 11$, a value which could occur by chance less than once

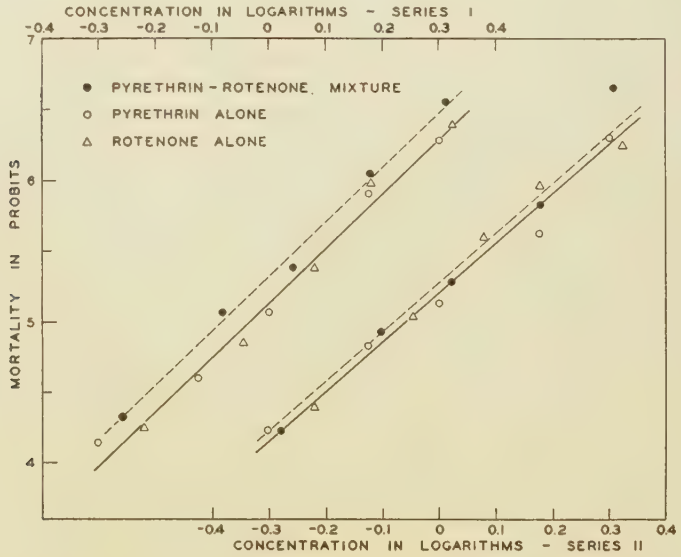

Fig. 6. Dosage-mortality curves for pyrethrin, rotenone and their combinations applied in alcoholic solutions to adult house-flies by a settling mist technique. All concentrations of rotenone have been multiplied by 6·03 to convert them to a pyrethrin standard. Data of Le Pelley & Sullivan (1936).

in 25 times, so that this mixture, containing 84·16 % of pyrethrin, was more toxic than would be expected from the potency of its ingredients. For series II, $M = -0{\cdot}0184 \pm 0{\cdot}0232$, a difference which could easily occur by chance, so that this mixture, containing 94·10 % of pyrethrin, showed no increase in toxicity over that expected from equation (5). These percentage compositions were determined from the relative weights of the two active principles in mg. per c.c., and with minor discrepancies were constant within each series. Since the toxicity of solutions containing both ingredients was greater at certain proportions of the two constituents

than could be predicted from the dosage-mortality curves for rotenone and pyrethrin alone, there was definite evidence of synergism, and these mixtures of rotenone and pyrethrin could not be classed under similar joint action as implied originally by Le Pelley & Sullivan (1936) but disputed by H. H. Richardson in a footnote to the original paper.

Synergistic action

Synergism forms a third type of joint action, characterized by a toxicity greater than that predicted from experiments with the isolated constituents. When the constituents of a mixture act independently, its toxicity does not depend upon the relative proportions of the components but only upon their inherent potencies. Synergistic action, in contrast, also involves the ratio of one component to the other. At the seat of action in the organism the two ingredients react with the living tissue as if they formed a single compound of enhanced toxicity. One would expect, therefore, that even if they differ both in potency and in slope when tested apart, the dosage-mortality curve for a given mixture would resemble that for a single poison when plotted in terms of logarithms and probits. No changes in slope would be expected which do not occur in the curves for the separate components. Any more inclusive formulation which will account for variations in the relative proportions of the ingredients must be consistent, therefore, with the basic dosage mortality curve when the ingredients occur in a fixed ratio.

The opposite of synergism is antagonism, which is characterized by a smaller toxicity than would be expected if the ingredients acted independently. One component interferes with the action of the other. Antagonism has been studied much more extensively than synergism, especially in its relation to pharmacology, and various mechanisms have been proposed to account for the quantitative relations in specific cases, theories which have been reviewed recently by Clark (1937). These are not of such a nature that they can be used in reverse form as an explanation of synergism except in special cases. Judging from the specificity of the relations in drug antagonism, no blanket equation for synergism is likely to hold for all possible ratios between two constituents. Yet within a restricted range, phenomena which probably differ in essential detail and complexity may show useful and striking similarities which invite generalization.

The basic data for a quantitative study of synergism consist of a series of dosage-mortality curves determined under comparable conditions, each curve representing a mixture in which the ingredients occur

in a single fixed proportion. In the simplest case the rectified curves are parallel within the limits of the experimental error and can be fitted with a single combined regression coefficient. The effectiveness of each mixture is determined from the equation of its curve in terms of the dosage producing a given mortality, such as the LD50, although the precision is improved if the mortality used for this purpose is the weighted mean probit for the series of curves.

Only two factors now remain, an equally effective dosage for each mixture and its percentage composition in terms of the essential ingredients. Two methods may be proposed for relating these variables. The more direct is to plot in terms of logarithms equitoxic dosages of mixture (the dependent variable) against the percentage in each of the more active ingredient (the independent variable). Although the interrelations of the two ingredients in such a diagram are relatively involved, it preserves the distinction between cause and effect inherent in the design of the experiment, which is an advantage statistically. It is also a more convenient form for later practical use. In several cases this relation has proved to be rectilinear over a useful range of compositions, so that within this range the results could be fitted by the equation

$$\log D_C + i' \log Q' = L, \qquad \ldots\ldots(8)$$

where D_C is the dosage of mixture $(A + B)$ producing a given kill, Q' is the percentage of the more active ingredient in the mixture, i' is the slope of the line, and L is a constant dependent upon the level of mortality used in fitting the equation. This expression has the further advantage that it can easily be extended to include three constituents or more. Then D_C is the dosage of all three components which is related to the percentages of the two more active ingredients, Q_A' and Q_B', by a partial regression equation

$$\log D_C + i_A \log Q_A' + i_B \log Q_B' = L, \qquad \ldots\ldots(8a)$$

where i_A and i_B are the partial regression coefficients showing the net relation between the total dose of mixture and the percentage composition of ingredients A and B in logarithms.

The left side of equation (8) may be looked upon as a modified or adjusted expression of dose. For any given mixture $i' \log Q'$ will be constant at all dosages and as a constant added to each $\log D_C$ it merely shifts the dosage-mortality curve for the mixture horizontally without altering the slope. When the dosage-mortality curves for the original mixtures are parallel, this characteristic can be used to test the validity of equation (8) by computing adjusted values for all dosages of each mixture and plotting the observed mortalities in probits against the

logarithms of the adjusted values. All observations in the range from which the adjustment has been derived should fall about a single straight line defined as

$$Y = a' + b\,(\log D_C + i' \log Q'). \qquad \ldots\ldots(9)$$

The regression coefficient b is the combined value determined from the original dosage-mortality curves and a' is computed from the average probit mortality $\bar{y}$ used in solving equation (8) as

$$a' = \bar{y} - bL. \qquad \ldots\ldots(10)$$

The fitted curve represents the effectiveness of any combination within the percentage limits covered by the straight lines of equations (1) and (8).

In original dosage units the relation between the total active material $(A+B)$ and the more active ingredient (A) may be written

$$(A+B)\,A^i = L', \qquad \ldots\ldots(11)$$

where $i = i'/(1-i')$ and L' is the antilogarithm of $L/(1-i')$. On this hypothesis the toxicity of a combination of two poisons which act synergistically is determined by the product of their sum multiplied by some power of the more active ingredient. Since the expression provides no lower limit for A, it is undoubtedly restricted to concentrations above a certain minimum. Otherwise a mixture containing only a trace of A would be less toxic than B alone, which is obviously untrue.

An alternative procedure is to plot for equitoxic dosages of each mixture its content of one ingredient against its content of the other. The dose of the combination is multiplied in turn by the proportion of each ingredient to obtain the amounts of A and B which in combination produced a constant effect. When plotted on cross-section paper, preferably in logarithms, each coordinate is restricted to a single component and is independent of the other. Since the relations between them are direct, this mode of analysis is preferred on toxicological grounds although both coordinates are then subject to errors of sampling. If the observations can be fitted by a straight line, its equation has the form

$$\log B + i \log A = \log J, \qquad \ldots\ldots(12)$$

where A and B are dosages of two poisons which in combination have a given toxicity, i is the slope of the line and J is a constant dependent upon the mortality used in fitting the equation. This expression is not equivalent to equation (8), although they may be indistinguishable when one component is always a small fraction of the other. It is restricted to a limited range of dosages for the same reasons as apply to equation (11).

This limitation can be avoided only by including a third parameter in the equation analogous to a minimal dosage for the smaller ingredient.

If A is the more active ingredient whose proportion in certain mixtures approaches zero, then

$$\log (A+K)+i \log B=\log J, \qquad \ldots\ldots(13)$$

where K is a constant which can be determined approximately by assigning various values to K until one is found by trial and error such that $\log (A+K)$ plotted against $\log B$ is a straight line with slope i. The log dose of B alone can be included with the mixtures in solving equation (13). After it has been computed in terms of logarithms, equation (13) can be transformed to

$$(1+k_1 A)\, B^i=k_2, \qquad \ldots\ldots(13a)$$

where $k_1=1/K$ and $k_2=J/K$. In this form it is a counterpart to that proposed by Gaddum (1937) for drug antagonism.

Synergism between a phenol and oil. As a numerical example the experiments of Kagy & Richardson (1936) on the toxicity of 2–4 dinitro-6-cyclohexylphenol (component A) and petroleum oil (component B) have been computed with the above equations. Component A, which is practically insoluble in water, was dissolved in the oil in proportions varying from 0·1 to 5 %. These basic solutions or mixtures were emulsified and sprayed on eggs of *Lygaeus kalmii* Stål. in the laboratory at three different concentrations of dilute emulsion. The relevant data are given in the first four columns of Table I. Both the number of eggs exposed and the percentage kill have been corrected for mortality in the untreated controls (4·1 %) by equation (2) or its equivalent.

The first step was to compute the dosage-mortality curve for each mixture. Unfortunately, the authors did not measure the dosage of $A+B$ deposited by each spray but only its concentration in the spray emulsion. Unless the one were directly proportional to the other, the concentration in the spray tank would not be a suitable measure of dosage and when transformed to logarithms would not lead to rectilinear dosage-mortality curves as, indeed, proved to be the case. Cressman & Dawsey (1934) have published data showing that the oil deposited upon camphor leaves was proportional to the logarithm of its concentration in the dilute emulsion, both in field and in laboratory experiments. For laboratory sprays the deposit y in mg. per sq. cm. of leaf surface could be represented by the equation: $y=0{\cdot}027+0{\cdot}042 \log x$, where x is the percentage concentration of oil in the spray emulsion. This equation has been "borrowed" to compute the expected dosages of insecticide in mg./100 sq. cm. corresponding to the 1, 2 and 3 % emulsions applied by Kagy & Richardson. When these computed dosages were transformed to logarithms, the probit kill could be plotted against them as a series of

Table I. *Combined action of 2 4 dinitro-6-cyclohexylphenol and petroleum oil sprayed in emulsions against eggs of a plant bug* (Lygaeus kalmii *Stål.); data of Kagy & Richardson* (1936) *analysed as described in the text. Dosages are in mg. of mixture*/100 *sq. cm. of surface*

Concentration of Phenol in oil mixture %	Concentration of Mixture in spray %	No. of eggs	Net kill %	Log dose x	Probit kill y	b	Log LD 79·6 for mixture	Log LD 50 in combination for component: Phenol	Log LD 50 in combination for component: Oil
0	1	240	6·5	0·43	3·49	7·16	0·749 ± 0·007	—	0·637
	2	479	40·1	0·60	4·75				
	3	479	58·7	0·67	5·22				
0·1	1	240	9·9	0·43	3·71	7·71	0·694 ± 0·006	−2·419	0·581
	2	479	59·7	0·60	5·25				
	3	479	72·3	0·67	5·59				
0·5	1	288	30·1	0·43	4·48	7·41	0·617 ± 0·006	−1·796	0·502
	2	479	73·7	0·60	5·63				
	3	479	90·4	0·67	6·30				
1·0	1	288	58·6	0·43	5·22	7·46	0·511 ± 0·008	−1·601	0·394
	2	384	94·0	0·60	6·55				
	3	288	97·22	0·67	6·91				
2·0	1	288	81·2	0·43	5·89	6·38	0·431 ± 0·010	−1·380	0·310
	2	384	97·13	0·60	6·90				
	3	288	99·65	0·67	7·70				
3·0	1	288	86·8	0·43	6·12	8·32	0·388 ± 0·012	−1·248	0·262
	2	384	99·48	0·60	7·56				
5·0	1	240	96·66	0·43	6·83	—	0·294 ± 0·022	−1·119	0·160

parallel dosage-mortality curves (Fig. 7) with a combined slope, b_C, equal to 7·392 ± 0·241. The combined slope has been used in estimating the log LD50 and other measures for each mixture.

Two alternatives are available at this stage of the analysis, the first relating the dosage of mixture directly to its percentage composition and the second separating each combined dosage into its two components, which are then related to each other. Both methods have been tested. As the basic data for the first, the toxicity of each mixture was measured from its curve in terms of the log dose which killed a specified percentage of eggs. The mortality having the highest precision for this purpose was the weighted mean probit of all mixtures containing from 0·5 to 5·0 % of the phenol component, 5·83 probits or 79·6 %. The log doses corresponding to this value are shown graphically by the intersections of the dosage-mortality curves in Fig. 7 with the lower horizontal line; in Fig. 8, each has been plotted against the percentage (in logarithms) of the phenol component dissolved in the oil. Except for the lowest concentration, the relation of the two variables could be fitted adequately by a straight line. The discontinuity between mixtures containing 0·1 and 0·5 % of *A* indicates the type of departure which may be expected as the proportion

of the more active ingredient approaches zero. Unless this departure can be shown to have a unique biological significance, such as is postulated for the curves in the first part of the present paper, the line fitted to the larger concentrations by equation (8) should be viewed as a useful approximation. In computing this approximation each observation has been weighted by the reciprocal of its variance, giving

$$\log D_C + 0{\cdot}307 \log Q' = 0{\cdot}521.$$

From this equation, the logarithm of the adjusted dose

$$(\log D_C + 0{\cdot}307 \log Q')$$

has been computed for the individual observations with each mixture.

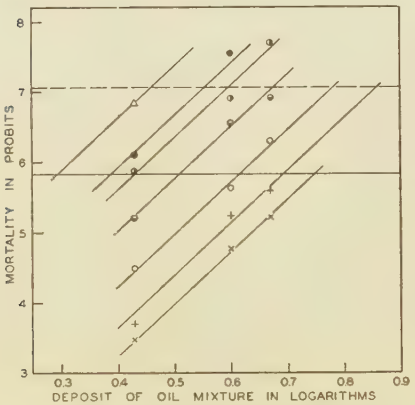

Fig. 7.

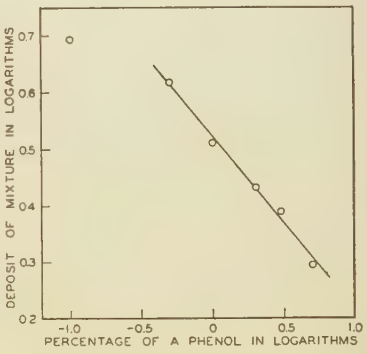

Fig. 8.

Fig. 7. Dosage-mortality curves for emulsions of oil containing 0, 0·1, 0·5, 1, 2, 3 and 5 % respectively of a nitro-phenol, reading from below upwards; based on the data of Table I. Intersection of the curves with the lower horizontal line gives log LD 79·6 (5·83 probits); with the upper horizontal line log LD98 (7·054 probits).

Fig. 8. Relation of dosage giving a mortality of 5·83 probits to the percentage concentration of a nitro-phenol in oil, as determined from the curves in Fig. 7.

When the probit mortalities were plotted against them, all values determined with mixtures containing 0·5 % of A or more fell about the expected curve computed by equation (9):

$$Y = 1{\cdot}977 + 7{\cdot}392\ (\log D_C + 0{\cdot}307 \log Q').$$

The results are shown in the lower curve of Fig. 9, and the observed mortalities were clearly consistent with the calculated line except as noted.

The relation of the oil deposited to its concentration in the spray has been computed from the evidence of another experiment. The estimated

dosages, therefore, are more or less hypothetical and not convenient for practical use in insect control. This difficulty has been avoided by plotting against the percentage of the phenol component (A) dissolved in the oil the percentage concentration of oil solution ($A+B$) in the dilute spray emulsion that killed 98 % of the eggs (Fig. 10 A). The curve was computed from equation (8), given above, by adjusting the constant L (0·521) to the higher level of mortality. For 98 % kill, $L = 0{\cdot}521 + \frac{7{\cdot}054 - 5{\cdot}830}{7{\cdot}392} = 0{\cdot}687$

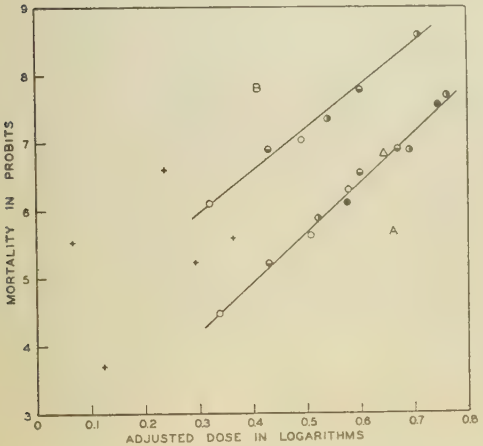

Fig. 9.

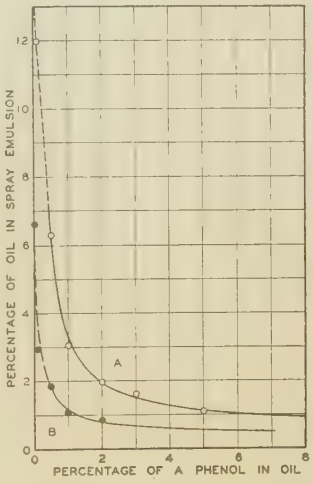

Fig. 10.

Fig. 9. Mortality as a function of the adjusted dose of oil and a nitro-phenol; A, eggs of *Lygaeus kalmii* Stål; B, overwintering *Aspidiotus perniciosus* Comst. The symbols have the same significance as in Fig. 7.

Fig. 10. Concentration of a nitro-phenol and oil required to kill 98 % of eggs of a plant bug (A) and of overwintering San Jose scale (B).

and $\log D_C = 0{\cdot}687 - 0{\cdot}307 \log Q'$. The deposits were first computed for values of Q' between 0·5 and 10 % and then transformed to percentages of oil in the dilute emulsion by reversing the equation used in estimating deposits from percentages. The observed values in Fig. 10 represent the intersections of the curves in Fig. 7 with 7·054, the probit for 98 % kill.

The data have also been analysed by the second procedure, in terms of the separate constituents A and B. For this purpose the LD50 for oil alone and for each mixture was multiplied in turn by the proportion of A and of B to obtain the amount of each ingredient which in combina-

tion killed 50 % of the eggs. In Fig. 11 the phenol component (A) has been plotted against the oil (B) in terms of logarithms. By omitting the mixture containing 0·1 % of A and the value for B alone (represented on the abscissa of Fig. 11 as a vertical line), the data agreed reasonably well with equation (12) but these restrictions could be avoided by using its alternative, equation (13). To determine the constant K, log $(A+K)$ was plotted against log B for several assumed values varying from 0·02 to 0·04. It was evident on inspection that the relation between the two

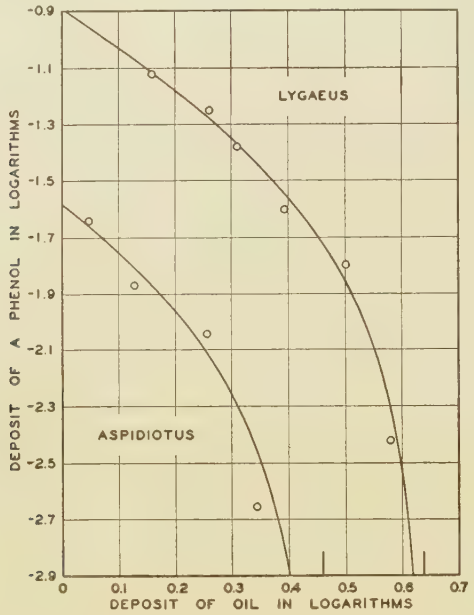

Fig. 11. Relation between dosages of a phenol (A) and oil (B) which in synergistic combination produced a kill of 50 %. The equation for the upper curve is $(1+25{\cdot}6A)\ B=4{\cdot}29$ and for the lower curve $(1+66{\cdot}7A)\ B=2{\cdot}73$. The vertical lines on the abscissa indicate the toxicity of oil alone, which the curves approach asymptotically.

variables was essentially rectilinear when K equalled 0·035 or 0·040. The regression coefficient (i) for $K=0{\cdot}035$ was 1·07 and for $K=0{\cdot}040$ it was 0·98, both near enough to unity that K was determined by interpolation as 0·039 so that i should be equal to 1·00. Hence the ordinate in Fig. 12 was adjusted to obtain a predetermined slope of 45° or $i=1$ and the equation of the line drawn through the observations was

$$\log (A+0{\cdot}039)+\log B=-0{\cdot}7764.$$

The straight line of Fig. 12 has been replotted in Fig. 11 after the necessary transformation.

The relation between the two components in units of mg. deposited per 100 sq. cm. of surface reduced to $(1+25{\cdot}6A)$ $B=4{\cdot}29$ for LD50 (equation (13a)). The same exponent ($i=1$) has been obtained in several cases of drug antagonism which have been computed by Clark (1937). If its occurrence is general in synergism as well, an adequate measure of the phenomenon must be sought in the two constants k_1 and k_2. Both k_1 and

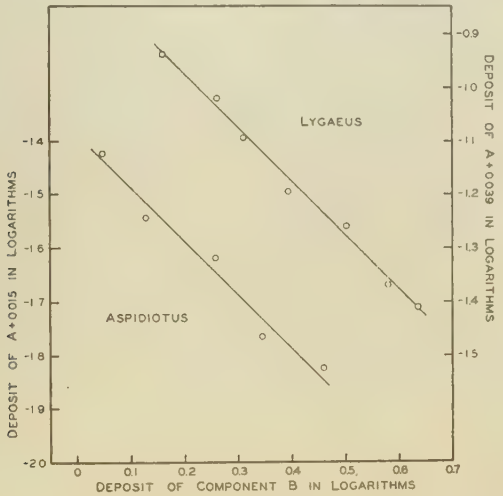

Fig. 12. Diagrams used in computing the equations of the curves in Fig. 11, which on these coordinates are straight lines with a slope equal to 1.

k_2 varied with the level of mortality at which the equation was solved. At the mean probit used in Fig. 8, for example, the above calculation gave $(1+19{\cdot}9A)$ $B=5{\cdot}56$ for dosages killing 5·83 probits. The product k_1k_2, however, was constant; at LD50, $k_1k_2=110{\cdot}0$ and at LD79·6, $k_1k_2=110{\cdot}6$. Any valid measure of the intensity of synergism should be constant at different levels of mortality within the same experiment, a criterion met only by the product k_1k_2, which may be adopted tentatively as a standard measure for synergism analogous to the ratio k_2/k_1 proposed by Gaddum (1937) for the intensity of drug antagonism. Since these mixtures followed the pattern for synergism, it is clear that they could not be described quantitatively in terms of independent action, as was demonstrated by Kagy & Richardson in their original paper (1936).

Kagy and Richardson tested the same materials against the San Jose scale (*Aspidiotus perniciosus* Comstock), although less extensively. When analysed in terms of the total dose of mixture and the percentage of component *A*, results were very similar to those obtained with *Lygaeus* eggs. All mortalities obtained with mixtures containing 0·5 % or more of the phenol component agreed with the expected dosage-mortality curve based on the adjusted dose: $Y = 4{\cdot}069 + 6{\cdot}330\ (\log D_C + 0{\cdot}363 \log Q')$. The line determined by this equation has been plotted with the experimental observations in Fig. 9*B*, the mixtures containing 0·1 % of component *A* diverging as before. When compared with its equivalent for *Lygaeus* eggs, the equation for 98 % kill, $\log D_C + 0{\cdot}363 \log Q' = 0{\cdot}472$, indicated that the scale was the more susceptible of the two insects, both to the mixture as a whole and specifically to the phenol ingredient. This equation has been transformed to the original experimental units and plotted in Fig. 10*B*.

The results have also been analysed in terms of the separate amounts of *A* and *B* which together killed 50 % of the scales. Agreement with equation (13) was not as close as that obtained with the eggs although the scatter of observations about the line (Fig. 12) was apparently random. The lower curve in Fig. 11 has been plotted from the equation of this line, $\log (A + 0{\cdot}015) + \log B = -1{\cdot}388$. In original units, $(1 + 66{\cdot}7\ A)\ B = 2{\cdot}73$ and $k_1 k_2 = 182$. The intensity of synergism increased about two-thirds in the San Jose scale over that found with *Lygaeus* eggs for the same materials and technique. This is not unexpected since three factors were involved, two poisons and the tissue of the insect. Any qualitative change in the last of these would be reflected in the quantitative relations of the phenomenon.

Synergism between the constituents of Derris root. Several prominent complex poisons and drugs are natural biological extracts in which the known ingredients may occur in very different proportions. Sometimes the standard procedure described here can be used in determining which of these ingredients have toxic value and the nature of their relation to one another. This can be illustrated by a series of tests reported by Tattersfield & Martin (1935) on the toxicity of Derris extracts to *Aphis rumicis*. Seven samples of root of different origin were extracted with ether, the dried resin dissolved in alcohol and the alcoholic solution diluted with a 0·5 % aqueous solution of saponin for application at various concentrations by a precision laboratory sprayer. The sprays were applied in six series of paired tests, each leading to two dosage-mortality curves based upon 5–7 concentrations apiece. For the present analysis the

percentage mortality has been determined from the counts of moribund and dead aphids two days after treatment and all curves have been recomputed.

The sensitivity of the aphids varied appreciably from one test to another. The two curves in each pair had the same slope within the limits of sampling error but the slope varied significantly from one pair to another. The level of susceptibility changed similarly between series, so that the four determinations of log LD50 for sample 5, for example, varied from 1·614 to 1·710, a difference which was significant statistically. For this reason the only comparisons between samples which were not confounded with variations in the susceptibility of the aphids were those within each series. Accordingly the log ratio of potencies (M) and its standard error have been computed for each pair. Using the weighted mean log LD50 for sample 5 as a standard, the M computed for each of the first four series was added in turn to this standard to obtain the log LD50 of samples 2, 4, 6 and 7, the standard error of each log LD50 being equal to the standard error of M, s_M, for the corresponding pair. The log LD50 for sample 1 was derived similarly from the estimate for sample 7 (series VI) and that for sample 3 from sample 1 (series V). By this procedure the toxicity in terms of the ether extract was determined for each sample on a strictly comparable basis. The results are given in the third column of Table II.

Table II. *Toxicity of ether extracts of Derris root applied as sprays to adult apterous female aphids* (Aphis rumicis)*; data of Tattersfield & Martin* (1935) *analysed as described in the text*

Sample no.	No. of dosage-mortality curves	Log LD50 of ether extract in mg./l.	Percentage composition of extract		Log LD50 in extract for component	
			Rotenone	Dehydro mixture	Rotenone	Dehydro mixture
1	2	1·397 ±0·032	27·7	37·7	0·839	0·973
2	1	1·601 ±0·027	20·7	29·0	0·917	1·064
3	1	1·400 ±0·050	22·3	43·0	0·749	1·034
4	1	1·640 ±0·026	13·0	24·8	0·754	1·035
5	4	1·666 ±0·010	9·8	28·5	0·659	1·121
6	1	1·413 ±0·042	32·4	29·0	0·923	0·875
7	2	1·422 ±0·018	35·5	28·0	0·972	0·869

The different toxicities of these ether extracts of Derris were due presumably to variations in their content of specific active principles. The chemical differentiation of these compounds is under active investigation and some of the ingredients which have been isolated chemically and tabulated by Tattersfield & Martin (1935) for these samples may later

prove to be secondary or mixed products having a reduced toxicity. With this limitation in mind, we may still determine which ingredients accounted most completely for the observed toxicity. Following the conclusions of the authors, three components were selected for study: (1) recrystallized rotenone, (2) dehydro mixture in the residual resin after removal of the rotenone, which will be treated here as if it were a single compound, and (3) all other constituents in the ether extract. The percentage composition of each sample in terms of rotenone and dehydro compounds respectively is given in the fourth and fifth columns of Table II; between them they amounted to 38–65 % of the total. To determine how completely they accounted for the toxicity of the ether extract, the net relation between the LD50 for total ether extract and the percentages of rotenone and dehydro compounds has been computed by equation (8*a*).

In computing this equation, the log LD50 for each sample was weighted by the reciprocal of its variance, so that the variation attributed to the differences in A and B as compared with the remaining constituents and error could be expressed in terms of χ^2. The results are in Table III,

Table III. *Analysis of variance for testing extent to which equation* (8*a*) *accounts for toxicity of ether extract in terms of recrystallized rotenone and dehydro mixture. By the present method of computation the sum of squares follows the* χ^2 *distribution and the probability integral P has been computed accordingly*

	All samples included			Sample 2 omitted		
Variance due to	Degrees of freedom	Sum of squares	P	Degrees of freedom	Sum of squares	P
Rotenone and dehydro components	2	206·06	<0·01	2	214·49	<0·01
All other ingredients and error	4	8·78	0·07	3	0·21	0·98

which shows that nearly all of the toxicity in the ether extract of these seven samples could be accounted for by the rotenone and the dehydro components, with the remaining ingredients probably non-toxic. Inspection of the plotted points showed one aberrant case of low toxicity, sample 2, and when this was omitted, the toxicity of the ether extract for the six remaining samples could be accounted for almost completely by the equation: $\log D_C = 2{\cdot}923 - 0{\cdot}452 \log Q_A' - 0{\cdot}556 \log Q_B'$, as shown in the right side of Table III. The differences between the observed log LD50's in Table II and the values estimated from this equation have been

plotted in Fig. 13 as differences from the computed curves for the log per cent of A and of B respectively. The agreement is all that could be expected with the exception of sample 2.

If the toxicity of these Derris extracts could be expressed in terms of two components, what was their relation to one another? This has been examined by determining the log concentration of A and B separately in each median lethal dose (Table II) and plotting the one against the other (Fig. 14). Again, sample 2 proved aberrant and when excluded, the remaining observations could be fitted adequately by a straight

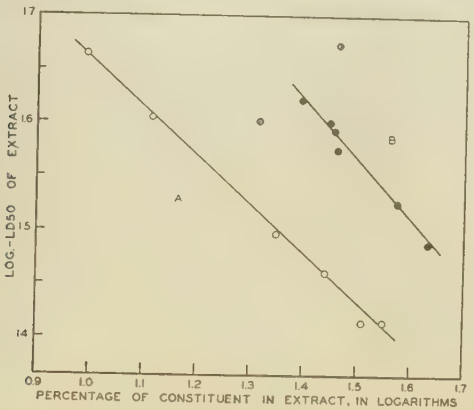

Fig. 13. Relation of median lethal concentration of ether extract of Derris root to its percentage composition of rotenone (A) and of dehydro mixture (B); data from Table II. Sample 2, represented by the shaded circles, has been omitted in computing the curves.

line (equation (12)): $\log B + 0{\cdot}820 \log A = 1{\cdot}660$. When reduced to two essential toxic ingredients occurring in about the same proportions, the toxicity of Derris root could be expressed most easily as a product function of the concentration of the two ingredients. The left side of the equation defines an adjusted dose which for log LD50 averaged 1·660, based on the milligrams of constituent per litre of spray mixture. When computed for each sample, this term varied from 1·632 to 1·667 except for the aberrant sample 2. Sample 2 was considerably less toxic than the others (1·815), indicating that it was out of line not because its full toxicity could not be expressed in terms of rotenone and dehydro mixture but rather because the estimate of this second component contained more inert matter than in the other samples. Rotenone exceeded

dehydro mixture in toxicity since its exponent in the equation $A^{0\cdot82}$ $B=45\cdot7$ was less than unity.

In contrast with the preceding example, there was no mixture in which one component occurred in a relatively small concentration, so that the relation between constituents could not be tested critically. Any one of several equations could be used equally well in the middle

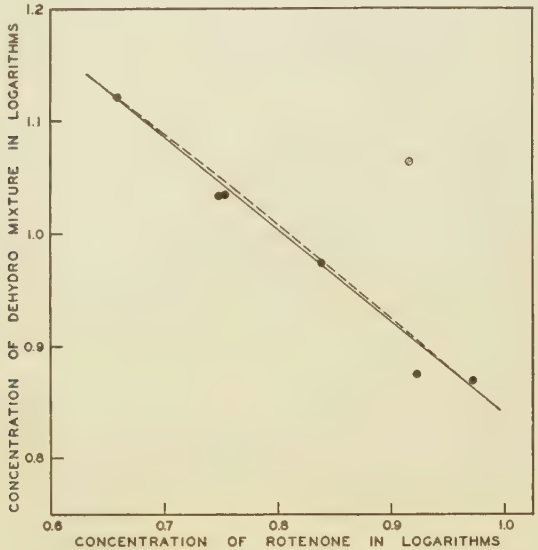

Fig. 14. Relation between concentrations of rotenone (A) and of dehydro mixture (B) which in synergistic combination produced a kill of 50 %. The equation for the straight, solid line is $A^{0\cdot82}$ $B=45\cdot7$, that for the curved, broken line is $(1+0\cdot714\,A)$ $B=56\cdot1$; sample 2, represented by the shaded circle, has been omitted in computing both equations.

range. The equation tested in the preceding example (equation 13)) has been computed with a regression coefficient of 1. Then

$$\log (A+1\cdot4)+\log B=1\cdot895,$$

or in the form of equation (13a), $(1+0\cdot714A)$ $B=56\cdot1$. The curve corresponding to this equation has been shown in Fig. 14 as a broken line for comparison with the solid line based upon equation (12). Except for sample 2 (1·903), the adjusted log LD50 based on equation (13a) varied from 1·719 to 1·756 and averaged 1·749. Synergism between rotenone and dehydro mixture was less pronounced than in the preceding example with the constant $k_1k_2=40\cdot1$.

Experiments with fumigants. The toxicity of poisonous mixtures which are ingested, injected or applied by contact sprays involves three factors: dosage, percentage composition and percentage mortality. The extension of these methods to mixed gases and vapours would involve four parameters, since the dosage of an air-borne poison depends upon two factors, concentration and exposure time. Although more experiments have been reported on mixed gases, perhaps, than on any other group of complex insecticides, the data have proved very refractory, largely because the dosage-mortality curve and the LD50 for each mixture have been determined in terms of the exposure time while percentage compositions have been necessarily in terms of relative concentrations. Effective progress in this field seems to depend either upon determining the concentration-time-mortality relation for each mixture, an involved and tedious task, or upon restricting all fumigations to a single standard exposure period and varying only the concentration to obtain the characteristic dosage-mortality curve for each mixture in a series. In the latter case the problem would be essentially the same toxicologically and statistically as the cases which have been described.

Summary

A quantitative analysis of the toxicity of drugs or poisons applied jointly requires that they be administered at several dosages in mixtures containing fixed proportions of the ingredients. From a study of the dosage-mortality curves for several such mixtures, preferably in comparison with equivalent curves for the isolated active ingredients, most cases of combined action can be classified into one of three types:

(1) The first type is that in which the constituents act independently and diversely, so that the toxicity of any combination can be predicted from that of the isolated components and from the association of susceptibilities to the two components. The coefficient of association can be measured experimentally and should be constant at all proportions of the ingredients. When high, the toxicity of the mixture is reduced. The form of the dosage-mortality curve has been examined for several hypothetical mixtures. Whenever the curves for the two constituents were assumed to differ in slope, there was a relatively abrupt bend in the curve for the mixture, the rectilinear segments above and below the break approaching in slope the values for the original constituents. This observation indicates that in homogeneous populations the slope of a dosage-mortality curve is of toxicological significance. Since the same numerical relations would be expected if a single poison were to have two independent lethal

effects within the animal, there is theoretical basis for fitting the linear segments of a dosage-mortality curve separately when a break occurs after transformation to probits and logarithms. This argument has been extended to time-mortality experiments to explain the smoothly concave curves characteristic of natural mortality.

(2) The second type of joint action is that in which the constituents act independently but similarly, so that one ingredient can be substituted at a constant ratio for any proportion of a second without altering the toxicity of the mixture. With homogeneous populations, dosage-mortality curves for the separate ingredients and for all mixtures should be parallel. Although by hypothesis the susceptibility to one ingredient is completely correlated with that to the other, mixtures in this category are more toxic than in the preceding class where association may vary from 0 to 1. The numerical relations have been illustrated by an experiment on the toxicity to the house-fly of solutions containing pyrethrin and rotenone. A mixture with a little less than four equitoxic units of pyrethrin to one of rotenone agreed closely with the definition but one in which the ingredients were about equally balanced showed a significantly greater toxicity than expected on the hypothesis of independent action, indicating the presence of synergism.

(3) Synergism forms the third type of joint action, characterized by a toxicity greater than that predicted from studies on the isolated constituents. It is the reverse of antagonism, which has not been considered directly. Two methods are proposed for the analysis of synergism. The more direct is to relate equitoxic dosages of mixture to its percentage composition in terms of the more active ingredient. When both are in logarithms the relation is linear over a useful range of compositions. This procedure preserves the original structure of the experiment, can be extended readily to three or more ingredients and leads to a convenient practical result. Theoretically it is less satisfactory than a second method in which for equitoxic dosages of each mixture the content of one ingredient (A) is related to the content of the other (B). The equation which satisfies this relation most completely is $(1+k_1 A)\ B^i=k_2$, where the three constants are computed from the experimental data. When the exponent i is equal to 1, only two constants need be determined and their product, $k_1 k_2$, is proposed as a measure of the intensity of synergism.

The synergism between a nitro-phenol and petroleum oil has been computed by both methods. For mixtures containing from 0·5 to 5 % of the nitrophenol, the deposit of mixture (D_C) killing 98 % of the eggs of a plant bug could be expressed adequately in terms of the percentage of the

phenol (Q') as $\log D_C = 0{\cdot}687 - 0{\cdot}307 \log Q'$, for 98 % of overwintering San Jose scale as $\log D_C = 0{\cdot}472 - 0{\cdot}363 \log Q'$. All observations, including those for a 0·1 % mixture and for oil alone which were omitted in the first method, could be fitted satisfactorily in terms of the separate ingredients. For plant bug eggs at LD50, $(1+25{\cdot}6A)\ B = 4{\cdot}29$ and for San Jose scale $(1+66{\cdot}7\ A)\ B = 2{\cdot}73$; in both cases $i = 1$ and the intensity of synergism 110 and 182 respectively.

The full procedure has also been applied to the constituents of seven samples of Derris root. One sample gave an unaccountably low toxicity and was omitted. The log LD50 of ether extract for the remaining six was related to the percentage composition of two components in the extract, rotenone (A) and dehydro mixture (B). Since the toxicity of extract could be expressed almost entirely in terms of these particular two constituents, they were then related to each other by the second method. None of the samples contained a very small proportion of one ingredient, so that several equations were equally applicable, one of them being $(1+0{\cdot}714A)\ B = 56{\cdot}1$, from which the intensity of synergism was 40.

The problem of measuring synergism in fumigants has been discussed briefly.

REFERENCES

Bliss, C. I. (1935). *Ann. appl. Biol.* **22**, 134, 307.

—— (1937). *Ann. appl. Biol.* **24**, 815.

—— (1938). *Quart. J. Pharm.* **11**, 192.

Boyce, A. M. (1931). *Mon. Bull. Calif. Dep. Agric.* **20**, 682.

Clark, A. J. (1937). *Handb. exp. Pharmak.* **4**. "General Pharmacology." Julius Springer.

Cressman, A. W. & Dawsey, L. H. (1934). *J. agric. Res.* **49**, 1.

Gaddum, J. H. (1933). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 183. His Majesty's Stationery Office.

—— (1937). *J. Physiol.* **89**, 7 *P*.

Hough, W. S. (1934). *J. agric. Res.* **48**, 533.

Kagy, J. F. & Richardson, C. H. (1936). *J. econ. Ent.* **29**, 52.

Le Pelley, R. H. & Sullivan, W. N. (1936). *J. econ. Ent.* **29**, 791.

Murray, C. A. (1938). *Soap*, **9**, Feb. p. 99.

Tattersfield, F. & Martin, J. T. (1935). *Ann. appl. Biol.* **22**, 578.

(*Received* 12 *January* 1939)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held at 2.35 p.m. on Friday, 17 March, in the Metallurgical Lecture Theatre of the Imperial College of Science and Technology, London, the President, Mr C. T. Gimingham, in the Chair.

Discussion on Testing and Diseases of Seeds

The following papers were read:

I. The production, handling, testing and diseases of seeds. By C. C. Brett, M.A.

II. Notes on seed transmission of *Phoma lingam* in relation to dry rot of swedes in Scotland. By R. W. G. Dennis, Ph.D.

III. Notes on *Pullularia pullulans* in Ryegrass seed and seed-testing methods as affecting detection of certain seed-borne diseases. By Mary Noble, Ph.D.

IV. Hybridization in *Brassicae* and the occasional contamination of seed stocks. By V. McM. Davey, Ph.D.

V. Modern methods of seed dressing. By W. R. Dillon Weston, M.A.

I. THE PRODUCTION, HANDLING, TESTING AND DISEASES OF SEEDS

By C. C. Brett, M.A.

Official Seed Testing Station for England and Wales, Huntingdon Road, Cambridge

The title of this paper suggests, at once, a consideration of seed as an article of commerce, and it is from this angle that I propose to approach the subject. The subject is so vast that in the relatively short space of time at our disposal it will be possible to discuss only broad outlines, and attention must be focused principally upon the more important agricultural and horticultural seeds passing through the usual trade channels in this country.

In the production, handling and testing of seeds, is summed up the activities of grower, merchant and seed analyst. In some instances, grower and merchant will merge into one, but for the most part the function of each is distinct, and each is mutually dependent. The final result of the combined activities of these three interests is seen in the normal trade in seeds.

Seed production

For the purpose of this symposium, I propose to discuss production from the economic rather than from the cultural aspect.

In this country, seed production, in its broadest sense, is in part organized and in part almost fortuitous. Organized production is exemplified by a contract system. Under this arrangement the farmer agrees to grow a specified acreage, the merchant supplying the seed and agreeing to take the seed produced from this acreage, provided that it is of good marketable quality. A proportion of the seed of the various cereals, seed of special strains of grasses and clover, most of the root-crop seeds and practically all vegetable seeds are produced in this way.

A direct contract of this kind is not the invariable rule, as sometimes a seed merchant will arrange with another merchant, either in this country or abroad, to place the necessary contract with the grower. In any case, it is usual for the merchant to reserve the right to inspect and rogue the growing crop.

The development, within comparatively recent years, of seed growers' associations, has already begun to influence to a certain extent the general aspect of seed production and marks a step towards organized production of a kind different from that already discussed. The original associations of this kind had as their primary aim the organization of the production of seed of certain local strains of red clover. They aimed at ensuring that purchasers of the seeds certified by them should obtain true and reliable stocks. This necessitated a system of annual crop inspections by competent judges of the strains concerned. Growers joining the associations were entitled to a certificate in respect of each crop examined and approved, and in some cases the association undertook to market the seed certified, whilst in others the members were at liberty to dispose of the seed themselves. More recently still, at least two associations have undertaken to grow certain of the Aberystwyth strains of clovers and grasses.

The Essex Seed Growers' Association is probably the largest, or at least has the widest interests and provides an excellent example of what can be achieved by such an organization. The members of this association now grow for certification, Essex late-flowering and Essex broad red clover and Aberystwyth seeds. As an indication of the growth of this association, it is interesting to note that in 1933 nine crops, totalling 66 acres, of late-flowering red clover were inspected, and that in 1938 the total acreage inspected was 336½, representing 46 crops, actually a slight decrease compared with 1937. Acreages approved and certified in 1938 were 120½ of Essex late-flowering red, 102 of Essex broad red, and 68 of Aberystwyth cocksfoot, timothy, perennial ryegrass and white clover. In the near future, it is anticipated that the association may expect even larger acreages.

Of the other associations, the Montgomery, Hereford, Suffolk and Cornish are at present concerned with local strains of red clover only, and the South-Western association grows Aberystwyth seeds.

The associations already referred to are purely local in operation, and the only scheme of a national character is the Wild White Clover Certification Scheme. This scheme came into operation in 1930 and was prepared by the National Farmer's Union and the Ministry of Agriculture. It is open to all growers of wild white clover in England and Wales and its object is twofold, namely, to assist the grower of suitable seed to obtain a market and a fair price for his produce, and at the same time to

afford a measure of guarantee to the buyer that he is getting the type of seed he requires. Two types of pasture are eligible for recording: (*a*) old pasture, that is, fields that have been under grass for 10 years or more, and (*b*) fields that are "once-grown" from seed saved from old pastures.

Some of these attempts at organized production are still in their early stages, but it is evident that there is a fairly widespread desire to secure to the producer of valuable strains of seeds the differential price that he is entitled to expect for better and more adaptable seeds and to guarantee to the buyer and user that he will get an article well suited to his purpose. It may well be, in the future, that the activities of such associations will exert a considerable influence upon the general trade in seeds.

Unorganized production of seed is dependent upon the open market. In the absence of a prearranged outlet for the seed, the farmer grows a seed crop without reference to the state of the market and not necessarily with a fixed plan for the disposal of the ultimate product. When the seed is threshed, it is offered on the open market and the merchant pays the market price ruling at the time. Generally speaking, seed of all the home-produced clovers and grasses is marketed in this way—with the exception of the special strains already alluded to—and a considerable proportion of the seed of cereals reaches the merchant through this channel. In most seasons there will also be a number of crops of peas and of kale, which, although sown with the primary intention of being used in the green state, may eventually be harvested for seed. In the case of peas, this may be due to an unfavourable market for green peas and in the case of kale to the fact that the keep is ultimately found to be superfluous for one reason or another. From the point of view of general seed production, the practice of saving seed from such crops is not always desirable, as the stocks from which they have been grown may not necessarily be suitable for this particular purpose.

It is clear that under the "contract method", the wholesale seed trade is able to exercise some measure of control over the production of seed. It must, however, be fully appreciated that the maintenance of seed supplies, even under this system, is not a simple matter, especially when it is realized that for certain kinds of seed, if they are sown say in June of 1939, the seed crop will not be taken until the autumn of 1940 and the resulting seed will not be used by the agricultural community until the spring of 1941.

Under the "open-market" system, there is, of course, no control of production. When crops are grown for seed, with no prearranged market, then fluctuations of supply become more pronounced, and in consequence prices are apt to show considerable seasonal variation. The influence of climatic conditions upon prices will often be more pronounced than under the "contract" system.

Certain varieties of some of the root and vegetable seeds are grown regularly in this country for export, and certain seeds are grown abroad on behalf of British merchants for import into and for use in this country. Variations in weather conditions in this and in other countries and fluctuations in the quantities of seed held by seedsmen and therefore in requirements year by year, all exercise important influences upon annual figures of import and export.

Of the seeds normally required for use in the United Kingdom, we are able to produce only a certain proportion of our total requirements of some kinds and none at all of certain others. Seeds which can be and are produced within the United Kingdom include cereals, red clover (both early and late flowering), Dutch and wild white clover,

trefoil, sainfoin, perennial and Italian ryegrass, crested dogstail, mangold, sugar beet, garden beet, swede, turnip, kale, tares, garden pea varieties, broad and runner beans, cabbage, brussels sprouts, parsnip, celery, radish and parsley. We do not, however, produce all that is required for home consumption in every case. In some instances normal home production has always to be augmented by imports, in others, imports—except of special varieties or strains—are negligible, there even being normally sufficient surplus of home-produced seed available for export. Species whose seeds are home produced in only negligible quantities, or not at all, include alsike, lucerne, meadow fescue, the so-called "natural grasses", dwarf-beans and carrot.

Handling of seeds

Cleaning.

Seeds purchased by merchants from growers invariably need further cleaning before they are in a marketable condition, and this has resulted in the production of specialized and often ingenious machines of various kinds, some designed to remove a number of different impurities at one operation, others to deal with only one or two special impurities. A large modern seed-cleaning warehouse is a model of organization and demonstrates clearly the extent of one of the major expenses incurred by those who handle seed in bulk. In the cleaning of seeds, the aim is to remove as much of the contained impurities as possible, without removing uneconomic quantities of the seed itself.

Drying and storing.

Apart from cleaning, there are occasions when seed needs to be conditioned or to be dried before it can be sold or stored. Specialized machinery for the drying of seed in bulk is a necessity in the case of merchants who deal in certain classes of seed. It is especially important that seed to be stored for any length of time should be of a suitable moisture content. For each species there is a limit in this respect, above which seed cannot be stored without incurring the risk of relatively rapid loss of vitality. Successful storage of seed also entails provision for ensuring that losses from vermin and insect attacks are reduced as much as possible.

Dressing.

Many modern seed merchants, especially those who deal in cereal seeds, now make a practice of dressing seed prior to distribution with one of the seed disinfectants now available—usually the proprietary organic mercury dressings. The practice of pre-treating in this way has undoubtedly increased to a considerable extent of recent years, and it is not unlikely that such treatment before distribution will continue to be practised to an increasing extent by seed merchants.

Distribution.

The distribution of seed can be divided into two fairly clearly marked divisions: (*a*) the distribution of home-grown seed, and (*b*) the distribution of imported seed. Home-grown seed, as we have seen, will in some cases have been obtained by the merchants by the "contract" method and in others in the open market. In both cases a certain amount of bulking will often be necessary before distribution to other merchants or to growers. A large proportion of the seed bought in the open market

will, in the case of most kinds of seed, be disposed of locally. Imported seed is chiefly dealt with, in the first place, by a limited number of brokers and wholesale merchants. Distribution is first mainly to other wholesalers and thence to retailers or to growers direct. Some bulks of seed, no matter what the source of origin, will always pass through the hands of a number of merchants before being finally distributed to the consumer, but imported seed usually passes through more hands than does home-grown seed.

The sale of seed and seed analysis

The sale of seed in this country, whether it be from merchant to farmer, or farmer to farmer, is controlled by an Act of Parliament, the Seeds Act 1920, with its complementary regulations. Except in certain specified cases, all transactions involving seeds scheduled under this Act must be in accordance with its provisions, the most important of which is that which states that the seeds concerned may not be sold or offered for sale unless a guarantee as to percentage purity and percentage germination is given at the time of sale or of delivery of the seed. The enforcement of this Act has profoundly influenced the trade in agricultural seeds and now it can be said that sales of seed in this country are primarily upon the basis of known purity and germination capacity. Prior to the introduction of this legislation, a certain proportion of the seed sold throughout the country was, of course, sold upon this basis, but there was no compulsion to do so, and, more frequently, seed passed from merchant to merchant and from merchant to grower at prices fixed chiefly upon the appearance of the seed and not upon any ascertained facts concerning its attributes. With the introduction of control over the sale of seed, there arose, of necessity, a greater interest in seed analysis.

The testing, or analysis of seeds, has developed as the result of attempts over many centuries to determine the value of seed from the point of view of crop production. The earliest interests in this direction took the form of rites and ceremonies, from which it was expected to obtain forecasts of the success or otherwise likely to attend the sowing of certain seeds or to obtain pre-knowledge of the quality and yield of the crops themselves. From these rites there developed a more precise interest in the seed itself, until in more recent times, following on increased botanical knowledge, there emerged a comprehensive idea of the seed as a living entity.

An an ordered process based on scientific principles, the development of seed analysis dates from about 1869, when Prof. Nobbe was working in Saxony. From then onwards, systems of control of the sale of agricultural seeds were introduced into most European countries, into America and into a number of the Dominions, and, of course, seed-testing stations controlled by the States concerned, or by some official body, were brought into being. For instance, in 1871, the Danish voluntary system of seed control was initiated and finally taken over by the State, with a seed-testing station at Copenhagen. Seed-testing stations were opened at Zurich and Breslau in 1875, at Vienna in 1881, Hamburg in 1891, Wageningen in 1898, Munich in 1902 and Berlin in 1906.

In this country a number of seed firms had started seed-testing laboratories of their own, before there was introduced any state control of the sale of seeds, and a privately owned seed-testing station, open to use by the trade, was started in London, at Wood Green, as early as 1890. In addition, facilities for the testing of seeds were provided at the British Museum, under Dr Carruthers, and the Royal Agricultural Society of

England made arrangements for seeds to be tested for its members by the consulting botanist. In 1910, the United Kingdom Seed Control Station was started by Mr S. F. Armstrong, an unofficial station, started as the result of requests from certain sections of the seed trade, for increased facilities for the testing of seeds.

The first official station in the British Isles was opened at Dublin in 1900—now the Irish Free State Station. The Scottish station was opened in Edinburgh in 1914, on a voluntary basis until legislation was introduced later. The official seed testing station for England and Wales opened in 1917 in London, later moving to Cambridge, and a station to serve Northern Ireland was opened at Belfast in 1922.

The application of the results of seed analysis to normal trade in seeds was given a tremendous fillip in this country, during the last War. The Board of Agriculture, as it then was, as part of its scheme for furthering war-time food production, introduced a Testing of Seeds Order, which was ultimately consolidated in the Act of Parliament to which we have already referred.

This Act, as was the original Order, was designed primarily to protect the farmer from the danger of unknowingly purchasing and sowing seeds of low vitality and contaminated with noxious weed seeds. No attempt was made to force the farmer to use seed of a better quality than he was prepared to buy; the aim was to make it an obligation on the part of the seller to disclose certain essential facts, so that the purchaser could judge for himself the value of the seed with a fair degree of accuracy. The Act is applicable to all the principal kinds of cereals, grasses, clovers, root and vegetable seeds. It requires that a declaration as to the percentage germination and purity, presence of injurious weed seeds and other specific particulars shall be made in writing to the purchaser at or before the time of sale or delivery of the seed. The introduction of the Act has made it necessary therefore for all dealers in agricultural seeds to ascertain these essential facts concerning seed offered for sale.

The official seed-testing stations in the United Kingdom serve to provide facilities whereby the seed trade may obtain the information concerning seed necessary to enable them to comply with the provisions of this Act. In addition, the Ministry of Agriculture allows the maintenance of private seed-testing stations, by seed firms, but these laboratories may be used by the firms concerned only for the purposes of their own trade. Control of the work of these private licensed stations is maintained by a system of inspection.

In many countries throughout the world, there are restrictions on the sale or import of seed, either enforced by law or adhered to voluntarily, which entail the seller knowing and disclosing facts concerning the seed similar to those required in this country. There have, therefore, arisen in most countries state seed-testing stations, as well as privately owned laboratories run by seed houses, where the chief work performed is the determination of percentage purity and percentage germination of various kinds of seed.

Most seed-testing stations of an official character undertake to determine the moisture content of seed samples, to examine seeds for the presence of seed-borne plant diseases, to make special identification tests of certain difficult species, and to give general advice concerning seed problems.

In practically all countries the determination of the two major features—percentage purity and percentage germination—is carried out along somewhat similar lines, there being certain variations in some directions, due to especial national considerations.

Before discussing methods, it is desirable to emphasize that with respect to the determination of these features, the aim is to ensure that the results of the tests may be utilized with safety as a basis upon which to judge the value of seed, especially in discriminating between different bulks of the same kind of seed. Admittedly, the results of laboratory germination tests do not usually agree with tests made under field conditions and, from the grower's point of view, the criticism that such tests are of little value in assessing the probable germination in the field is valid up to a point. The faithful reproduction of field conditions in the laboratory is impossible of attainment, and therefore methods to determine germination capacity have been evolved which will allow of the conditions imposed being subject to control and also of being standardized. That uniformity and simplicity of method is desirable is apparent in this country, where, in addition to the official stations, there are some thirty-two private licensed stations authorized to test all kinds of seed, and some forty-six others holding licences of limited application. For the purposes of international trade in seeds, the International Seed Testing Association has adopted a comprehensive set of rules, to which all countries subscribing to the Association agree to adhere for the purposes of issuing international certificates.

The determination of the percentage purity has, for practical considerations, to depend upon the examination of a relatively small quantity of seed. This small test sample must be drawn from the bulk sample in such a way as to ensure, as far as possible, that it is truly representative. Various hand methods can be employed and there are a number of mechanical contrivances designed to perform this operation. In this country it is usual to employ the continuous halving method. Two test samples are drawn in this way, and from each is then weighed a definite quantity upon which a purity analysis is conducted. The weights of seed taken vary with different species—the larger the seed, the greater the quantity taken for the purity analysis. Considerable accuracy is desirable at all stages, as the amounts analysed are small and errors will be magnified when the final calculations are made.

Having obtained the weighed quantity of seed, all impurities are separated, leaving the pure seed, of which the sample purports to consist, in a separate heap. This separation is usually performed by placing the test sample upon a suitable glass slab and with a pointed spatula passing the seed in a stream from one side of the slab to the other, all impurities being moved to one side in the process. These impurities are then grouped thus:

Injurious weed seeds. These are species specified in the Seeds Act as being especially noxious.

Other weed seeds. Seeds of weed species other than those in the above group.

Useful species. Seeds of crop plants other than that of which the sample consists.

Inert matter. Obvious material, such as stones, grit, chaff, and vegetable debris; seed so broken or insect-eaten as to be incapable of germinating; light seed of grass species; empty clusters of beet and mangold; immature seed of carrot; sclerotia and spore masses.

The impurities having been identified and grouped, the groups are weighed, the percentage of each group determined and the percentage of pure seed calculated. The results of the two independent purity tests are averaged.

The purity analysis therefore discloses certain features:

(1) The total percentage of pure seed.
(2) The percentage of especially noxious weed seeds.
(3) The percentage of other kinds of weed seeds.
(4) The percentage of other kinds of crop seeds.
(5) The percentage of worthless and harmless material.

In addition it reveals the nature of the individual species within each group, though not the percentage occurrence of each individual. Actually, the Seeds Act necessitates the declaration of certain specified species, where they occur to more than a prescribed percentage, and therefore these species need to be separated and their percentage occurrence determined. In addition, the Act includes a special provision which makes it necessary on the part of the vendor to disclose the presence of dodder, if it occurs to more than a prescribed extent in certain clovers, in timothy and in flax. This entails the examination for the presence of seeds of *Cuscuta* spp. of relatively large quantities of seed.

A scrutiny of the individual species within the groups separated in the purity analysis will sometimes give an indication of the country of origin of the seed, as certain species of plants are characteristic of definite regions.

The purity analysis then gives other useful information, apart from the mere percentage of pure seed. It would be of even more value to the grower if the impurities present in a sample were indicated as representing so many seeds per pound of the sample, but few countries yet insist that this information should be available to the purchaser of seeds.

The purity analysis is by weight, the germination test by numbers, the latter being made upon the pure seed separated in the former. The germination test is devised to find the maximum percentage of seed capable of *complete* germination when placed under suitable controlled conditions.

The general routine in making the germination test is as follows:

A number of separate hundreds of pure seed—the number of hundreds depending upon the nature of the seed—are placed under conditions favourable to germination, and at intervals all seeds which have completely germinated are removed, counted and the number recorded. Intermediate counts of germinated seed are continued throughout the full period of test, but their frequency will vary with different species. In practice they are made as frequently as experience has shown to be desirable, bearing in mind that the seedlings to be counted must be advanced enough for it to be possible to discern clearly whether or not they are quite normal. At the last germination count to be made, the total number of seeds germinated out of each 100 is determined and the average of the sets is struck and this figure taken to represent the percentage germination of the sample. In addition, the number of dead seeds, abnormal seedlings, hard seeds or fresh seeds is noted and the totals checked against the original hundreds.

As a result of experience and of expedience it has been found that it is desirable to fix a maximum limit of time for each species for the germination test, so that it is now generally understood that a germination test shall continue for only a certain number of days, at the end of which time the test will be completed.

The intermediate counting of the number of seeds germinated is undertaken for two reasons:

(1) In order to simplify the actual process.

(2) To give further useful and usually valuable information concerning the seed, in addition to knowing the total percentage of seed capable of complete germination: this information is obtained by noting the percentage of seed germinated at each intermediate count, and this gives an indication of the speed of germination. To the grower such information is especially useful. It is usual to strike an average of the seed so far germinated at the count made at about half-way through the full period of test, and to consider this figure as representing the energy figure of the seed.

For each different kind of seed it has been found desirable to fix a minimum number of hundreds of seed which should be put to germinate, in order to ensure a reliable result. This has been determined as the result of experience, and bearing in mind difficulties of manipulation of material and economy of space. Concerning length of test there is variation between species, as some habitually germinate relatively rapidly, whilst others germinate much more slowly and therefore need a longer period in order to disclose their maximum capabilities.

When the final germination count is made, it is usual to scrutinize the remaining seeds and seedlings, and frequently further information can be obtained at this stage concerning the sample as a whole.

In clover species for instance, there are invariably seeds remaining at the end of the germination test which have not germinated and have not imbibed water. These are known as "hard seeds", and it is possible that in course of time such seeds would imbibe water and germinate, and in fact such is known to be the case, but the rate at which imbibition takes place varies with different species and from sample to sample of the same species, so that in this country it is usual to determine the percentage occurrence of these hard seeds and to report them separately. In other species, too, more especially in brassicas, seeds are encountered which remain ungerminated at the end of the test, but which are not dead. In these cases the seeds are best described as "fresh", and they are usually associated with new crop seed tested soon after harvest. In most cases they are probably seeds which are not fully "germinating ripe", but which in time would fully ripen and so be capable of germinating.

Abnormal seedlings of various types are frequently encountered especially amongst clover species, *Brassica* spp. and onions. It is desirable that seeds which produce seedlings of an abnormal character should not be classed as having germinated, and that for the purpose of determining the percentage germination, only those seeds which produce definitely normal seedlings should be taken into consideration. The criterion of germinability is the capacity of the seed to produce a normal seedling, and a normal seedling must possess a normal root with root hairs, an intact plumular bud and at least one normal cotyledon.

Concerning the types of abnormal seedling referred to, their occurrence can usually be associated with some defect in the seed itself. In the case of clover species, abnormal seedlings, when produced, are most frequently the result of internal injury to the seed, which it has sustained during the usual seed-cleaning processes. Some forms of abnormality can be ascribed to old seed. "Fresh" seeds in brassicas are indicative of recently harvested seed and abnormalities in these species are invariably associated with old seed.

The injurious effect of certain kinds of seed dressings is often reflected in the production of abnormal seedlings during the germination test. In graminaceous seeds, over-dressing with copper sulphate or formalin induces a type of abnormality, in which the radicle is so affected as to produce no primary roots, the plumule only developing. Under certain circumstances, over-dusting with organic mercury compounds results in the production of a particular and characteristic form of abnormal seedling.

The value of the germination test can be summed up as follows:

(1) The total percentage of seed capable of complete germination is disclosed.
(2) The vigour or speed of germination is indicated.
(3) In the case of some species it discloses whether the seed is fully "germinating ripe".
(4) If the seed has suffered physical, internal damage, or damage from chemical seed dressings, such damage is apparent from the germination test.

It has already been stated that to determine the percentage of germination, it is the practice to place the seeds under optimum conditions of growth. It has been found that different species have different requirements in certain directions, in order to obtain satisfactory germination results, so that for each kind of seed there has been evolved a set of conditions to which all samples of the same kind of seed are subjected, in making the germination test. In arriving at these sets of conditions, the aim has been to find a combination which will give the most regular, rapid and complete germination for the majority of samples of the same kind of seed.

For successful germination, under laboratory conditions, the following factors play an important part:

(1) Nature of the seed bed.
(2) Degree of humidity.
(3) Temperature.
(4) Aeration.

All so-called "standard" methods of germination are simply combinations of the first three of these, the differences in methods being reflected in variation of these factors. In practice, the nature of the seed bed is often a vital factor in controlling the degree of humidity, so that the two must be considered in relation to one another.

The actual apparatus commonly in use in making germination tests is probably familiar to everyone, as are the types of seed bed found to be most useful in seed-testing practice. In selecting materials for use as substrata, the chief considerations are water-holding capacity, the ease with which the material can be rendered sterile, and simplicity in manipulation. Sand is the most generally useful material, as it has the obvious advantages of being cheap and easy to sterilize, and enables a large proportion of the total surface area of the seed to be maintained in close contact with the source of moisture. In practice, sand is chiefly used for the larger seeded species, whilst for most small seeds filter paper proves to be most suitable. Humidity and temperature requirements of the different classes of seed cannot now be discussed in detail, but it must be pointed out that for different species variations of these factors are found to be necessary.

We have not the time to discuss methods in detail, nor to consider the finer points

in seed analysis practice, but this brief outline of general principles will serve to indicate the trend of modern practice.

It now remains to consider, very briefly, the interpretation of the results of seed analyses. For the merchant this is relatively simple. He buys and sells upon specific known attributes, such as percentage purity, germination, moisture content, bushel weight, etc.

For the grower the interpretation is somewhat different, as the results of seed analysis give no direct guarantee of productivity in the field. The grower's chief concern being with field results, he must take into account both purity and germination and assess the real value—or percentage of pure germinating seed. In conjunction with price, this figure will afford a reasonable means of comparison. To form some judgement respecting field performance, the speed of germination or energy figure is of most value, and if this figure is known and it is also appreciated that, in general, high laboratory germination suffers less proportional reduction in the field than does a low one, the grower should be in a position to assess the value of any given bulks of seed for comparative purposes. From the detailed information given in the purity analysis, the grower can form a reasonable judgement as to the suitability of a sample for his purpose, bearing in mind the nature and extent of the impurities in relation to the crop seed concerned.

Seed-borne plant diseases

The commercial seed analyst is usually in a position to determine the presence of only those diseases which can be recognized by examination with the unaided eye, but this information, although limited, could be of considerable value to both merchant and grower.

The determination of other diseases is usually outside the scope of the commercial analyst, but it should be within the province of all state seed-testing laboratories to report the presence of those diseases which are known to be seed-borne and of economic importance. There appears to be a growing interest, on the part of those who buy and sell seed, in the general problem of seed-borne diseases, especially in those diseases which can be controlled by the application of the modern seed disinfectants. But the fact that a number of seed-borne diseases can be recognized in seed samples with comparative ease, could be used to greater advantage by merchant and grower than is at present the case. The implication of this fact in the direction of disease control needs no elaboration.

Amongst the seed-borne diseases which may be encountered in commercial seed samples may be mentioned the following:

Tilletia Caries, bunt of wheat—by bunted grains and by adhering spores.

Ustilago Hordei, covered smut of barley—by smutted grain and by adhering spores.

Ustilago spp., smuts of oats—in oat samples—by adhering spores.

Claviceps purpurea, ergot sclerotia in rye, wheat, ryegrass, *Agrostis*, *Holcus* and timothy.

Helminthosporium Avenae, leaf spot of oats.

H. gramineum, leaf stripe of barley.

Tilletia decipiens, in *Agrostis*.

Aplanobacter Rathayi, in cocksfoot.

Septoria Apii, celery leaf spot or blight.
Phoma apiicola, *Phoma* root rot of celery.
Phoma lingam, dry rot and canker of swede and other brassicas.
Phoma Betae, in mangold and beet.
Ascochyta, pea spot.
Sclerotia of *Typhula*, in red and white clover and sainfoin.
Sclerotia of *Sclerotinia Trifoliorum*, in certain species of Trifolium.

The official stations in the United Kingdom at present report as a matter of routine the presence of only those diseases which can be recognized by the unaided eye, but they are prepared to examine samples for certain specific diseases upon request.

II. NOTES ON SEED TRANSMISSION OF *PHOMA LINGAM* IN RELATION TO DRY ROT OF SWEDES IN SCOTLAND

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Though not a cash crop, swedes form by far the most extensive root crop north of the Border; normally their acreage is about three times that devoted to potatoes. In most districts finger and toe, though present, is not a very serious disease, and the main losses attributable to fungi take the form of dry rot. The particular outbreak which led to the present investigation occurred in the Lothians, but heavy losses are also known to have occurred in the counties of Ross, Inverness, Aberdeen, Forfar, Fife, Perth, Dumbarton, Ayr and Wigtown. Its distribution is, therefore, not to be correlated with the rainfall, since it is at least as severe in the dry north-east as in the wet south-west.

So much work has been done on dry rot in the past that, as regards symptoms, development of the disease and its purely mycological aspects there is little that is new to relate. The chief question on which sharp differences of opinion are still possible is that of the immediate source of infection which starts an outbreak of dry rot in the field. It is well known that once a few mature roots have been attacked infection will spread readily, along the row or in a pit.

Previous workers have shown that *Phoma lingam* can be carried on swede and turnip seed, but with few exceptions they have concluded that such infection is insignificant in amount and plays little or no part in initiating outbreaks of dry rot in the field. According to these authorities the usual sources of such outbreaks are to be sought in infected soil, and in overwintering of the pathogen on infected debris or on susceptible weeds. In 1938 the attention of the Plant Pathology Service for Scotland was drawn to this subject as a result of reports of heavy losses due to dry rot in the swede crops of the Lothians. In this district, where mixed farming is the rule, swedes form the staple winter food for cattle and sheep. The typical Lothian practice is a six-course rotation, including only one Cruciferous crop which may follow either potatoes or oats. There is, therefore, no question of unusually rapid contamination of the soil with infected debris as a result of continuous cropping. On the other hand, the

prevalence of finger and toe indicates that this 5-year interval is not long enough to keep down the population of true soil fungi. In this district dry rot has been recorded on swede crops taken on freshly broken old pasture. It has also been recorded as prevalent where a long rotation amounting to as many as 10 years is practised.

It does not seem likely that the main source of dry rot infection in Scotland can lie in cruciferous weeds. Swedes are used as a cleaning crop and are kept reasonably free of weeds. Also the commonest weeds of arable land in Scotland are Persicaria and spurry. Runch and charlock are seldom seen among swede drills. Infection from hedgerow weeds seems unlikely since the disease appears scattered throughout a field and does not originate in the neighbourhood of hedges.

According to reports of farmers and college officers, dry rot is said to be getting worse each year, and statements have been made to the effect that swede growing in the Lothians will have to be abandoned in favour of some non-cruciferous root such as mangolds. It seems fairly obvious that the very bad season of 1938 was partly responsible for the severe outbreak of disease last autumn. It is the practice in the Lothians not to pit turnips but to cart in a few days' requirements and leave the rest in the field. Hence the crop is subject to excessive frost damage, and at the present time one sees in swede fields at least as much bacterial rot following frost injury as true dry rot. It is probable that the two rots have been confused by farmers and others who have made the extreme statements referred to. I shall be interested to hear members' views on the best way of minimizing spread of dry rot during winter storage.

When a farmer suffers heavy losses from disease there seems to be a natural tendency for him to blame the seed. In view of the numerous complaints received, a number of seed merchants have revived the question of the importance of seed-borne infection and have called for a reinvestigation of the problem.

In preliminary trials swede seeds were plated on malt agar, but this method gave unsatisfactory results owing to the presence of numerous saprophytes. More information was gained by germinating seedlings on damp filter paper in Petri dishes, 100 seeds per dish. Under these conditions *Phoma* infections as high as 2 % were recorded. Infected seedlings rotted and as many as forty pycnidia developed on the decayed radicle. Under these conditions, however, the seedlings were rather weak and it was difficult to maintain uniform humidity. Further experience has shown that the most satisfactory method of estimating infection is that recommended by Buddin, namely, to germinate the seed in the usual way on Jacobsen germinators. Fifteen seeds are as many as can conveniently be accommodated under a 2½ in. bell jar and, with the germinators used, this implies a total of 840 seeds in each experiment. Even with this fairly wide spacing of the seeds there is still the possibility of disease spreading from an infected seedling to the others on a pad. Hence, in estimating the degree of infection only one infected seedling is counted on each pad, so that the figures to be quoted should be regarded as minima. From Buddin's work it is known that pycnidia of *P. lingam* do not form readily in the dark, but it was not at first realized that full sunlight is essential for reliable results. At first the germinators were set up in a west window, but it has been found that satisfactory results are only obtained when they are moved to a south window and placed so as to receive the maximum amount of direct sunlight. Under these conditions pycnidia may develop in 5 days on the testa of a severely infected seed. Such seeds decay without germinating and pycnidia do not develop on the seedling in less than 12 days. Such seedlings usually develop a rot

which involves radicle, hypocotyl and eventually the cotyledons. Pycnidia form freely on all these organs and may readily be recognized under a hand lens by their copious purple exudate. If the testa is carried up on the cotyledons infection may originate there, as described by Cunningham, but usually the testa is not carried up and infection starts at the base of the hypocotyl or at the root tip. Development of brown markings or stripes on the hypocotyl is not a sufficient indication of infection with *P. lingam*. Such symptoms can be induced by *Alternaria brassicae* and even by *Cladosporium* sp.

Three seed samples of known origin may be quoted to indicate the order of the figures obtained. Of these no. 1 was from a stock of seed other samples of which had given heavily infected crops in 1938. Samples 2 and 3 were daughter seed to be used in 1939. The results are as follows:

Sample 1. Seedlings infected on roots only, 26; other infected seedlings, 8; total, 34 or 4 %.

Sample 2. Roots, 35; others, 14; total, 49 or 5·66 %.

Sample 3. Roots, 13; others, 2; total, 15 or 1·74 %.

As previously mentioned, these figures are arrived at by counting only one infected seedling on each pad. The actual number of infected seedlings present may be taken as about double these figures. It is, of course, arguable that although 5 % of the seedlings may bear *Phoma* spp. with purple exudate this is not sufficient to identify them as *P. lingam*. In admitting the justice of such criticism, one could adduce in self-defence that most modern authorities seem inclined to regard all the *Phoma* spp. with elongated spores about 5–6 by 1·5–2μ, listed on the brassica by early mycologists as falling under the general heading *P. lingam*. The spore measurements of the fungi obtained in our germinators fall within the rather wide range quoted for that species. The *Phoma* spp. present certainly appear to be pathogenic to the seedlings under the conditions prevailing in the germinators and some of them, at least, have yielded cultures which proved capable of initiating dry rot in healthy swede roots. It may be mentioned in passing that two distinct types of growth in pure culture can be obtained from the same pycnidium. The first is dark and rather greenish and spores readily; the second is brown and is almost sterile. Whether these two forms correspond to the mycelial and conidial strains isolated by Hansen from other imperfect fungi it is too early to say.

According to Hughes, 1 % infected seed is equivalent to one infected seed in every 3 ft. of drill. According to Buddin it means the sowing of 3000 infected seeds per acre. Assuming a distance of 2 ft. between the drills the latter estimate implies one infected seed per 2·63 ft. of drill. 5 % infection, therefore, means the sowing of an infected seed to every 7 in. of drill, or about one to every group of seedlings removed at singling. Such a figure is much higher than that recorded as usual in the Ministry of Agriculture's *Bulletin* on dry rot and canker. On the other hand, it is difficult to estimate the probable importance in the field of even 5 or 6 % infection. On the germinator an infected seed either rots or gives rise to an infected seedling which decays and apparently would not develop into an established plant. Moreover, even if infected seedlings became established, the chances are greatly in favour of their being cut down in singling when only one plant is left to each 10 or 12 in. of drill. Hence, the chances are greatly against the dry rot observed on a swede in autumn being due to infection of that particular plant from the seed at the time of germination. On the other hand, it is generally

considered that dry rot originates from infected debris lying in the soil, and it is evident that sowing of infected seed results in distribution of such infection foci very regularly over the soil of a turnip field. If seed-borne infection proves to be important it is probably in this manner that it will be found to be effective.

As regards the question of detecting seed-borne infection in routine seed testing, it appears that few difficulties should arise provided the necessary precautions are observed. The testing can be done in the usual way on Jacobsen germinators exposed to a good light. I understand that in current practice *Brassica* seeds are usually germinated between filter papers in germinators with glass doors. Under such circumstances pycnidial formation is likely to be reduced to a minimum and a very unreliable estimate of infection may result. According to the International Rules the final count is made after 10 days; from the point of view of seed infection this is quite inadequate. Pycnidia do not usually appear in less than 12 days on the seedlings and the samples should be kept for at least three weeks before the final counts are made.

If it is found that seed-borne infection with *Phoma lingam* has a significant effect on the keeping properties of the swede crop, then steps will have to be taken to eliminate it. As previously mentioned, a number of seed firms are already alive to this possibility and have sought advice on the subject. Owing to the thin testa and consequent delicacy of the swede seed, treatment with the standard wet or dry disinfectants is not altogether desirable. Although hot-water treatment may be effective it is hardly practicable at present on a commercial scale. The solution of the problem would seem to lie along the lines of more rigid inspection and selection of seed plants from the point of view of freedom from dry rot and canker.

III. NOTES ON *PULLULARIA PULLULANS* IN RYEGRASS SEED AND SEED-TESTING METHODS AS AFFECTING DETECTION OF CERTAIN SEED-BORNE DISEASES

By Mary Noble, Ph.D.

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The first record of *Pullularia pullulans* occurring on Ryegrass seed appears to be a note published by Hyde in the *New Zealand Journal of Agriculture* of May 1938. In this note he does not name the fungus but merely states that the fungus causing low germination of Italian Ryegrass in New Zealand is probably the same parasite as is commonly associated with Perennial Ryegrass. In October 1938 in the same *Journal* he published a fuller account under the title "Detecting *Pullularia* infection in Ryegrass seed crops". This is a semi-popular article and in it he states that "it is now generally recognized that the fungus responsible for the low germination of Ryegrass seed is *Pullularia*". The infected seed while within the glumes was indistinguishable from healthy seed but on removing the glumes the kernel was found to be shrunken and rather pink-brown in colour. Infected seed could, however, readily be detected by the use of the diaphanoscope; healthy seed should be bright and translucent but the seed infected with *Pullularia* was found to be dark and opaque. According to Hyde, fresh seed bears masses of pink or buff-coloured spores on and around the kernel. Infected seeds failed to germinate.

In December 1938 our attention was drawn to this question by a firm of seed merchants who had seen the above-mentioned articles and wished to know if the fungus was present on Ryegrass handled in this country. Samples of seed of *Lilium perenne*, and of mixtures of *L. perenne* and *L. Italicum*, were submitted for microscopic examination and *Pullularia* was found easily and readily.

Seeds which were opaque on the diaphanoscope were selected. It was found that the glumes were, in most cases, firmly stuck to the kernels and that these were mostly shrunken as Hyde described. But, where Hyde found masses of spores on the kernels of fresh seeds, we only found on these dried mature seeds little grains of rather powdery consistency, but these seemed to account for the glumes having been so firmly attached to the kernels.

When infected seeds were placed in a drop of water and examined microscopically the spores were seen to float off into the water in very great numbers. They were hyaline and oblong in shape, measuring roughly 12 by 2 μ. These spores failed to germinate either in hanging drops of water or on poured plates of any of the ordinary laboratory media, so that, at first, it was difficult to identify the fungus without having it in culture. Eventually, cultures were obtained from the mycelium present within the seed. These cultures grow very quickly, producing masses of pink or buff-coloured spores on the surface. *Pullularia pullulans*, being synonymous with the old species *Dematium pullulans*, has the property of producing spores which bud in the yeast-like fashion characteristic of the Saccharomycetes, and as the spores described proceeded to bud freely it seemed likely that this fungus was indeed *Pullularia*. So many of the pink spores are formed in the cultures that they very soon have the appearance of a bacterial slime on the surface of the medium. The hyphae are at first hyaline but soon they become opaque with thickened walls and are brown in colour; dark brown chlamydospores are formed more slowly.

All these features agree well with a description of *Dematium pullulans* in culture published by Bennett in *Annals of Applied Biology*, 1928. It seems fairly certain that we are dealing with *Pullularia pullulans*, but whether it is the cause of the death of the Ryegrass seed in this country and in Ireland, as Hyde has found to be the case in New Zealand, has yet to be demonstrated.

A few experiments have been carried out on the germination capacity of infected seeds. It was found that as a working hypothesis it could be assumed that the opaque seeds were infected with *Pullularia* and that most of the translucent seeds were free. It seemed, at first, that all the translucent seeds could be regarded as healthy but, later, it was found that some carried the fungus, although these were not so heavily infected as the opaque seeds. It must be emphasized, however, that when a sample of Ryegrass seed is examined on the diaphanoscope, it is not possible to distinguish at once the translucent and opaque seeds as there is a range from those which are quite light brown to those which are darker brown and so on to those which are almost black. There is a strong human element involved in selecting what are to be termed opaque and translucent seeds, so that any attempt to analyse results statistically is not entirely successful.

In the first experiment fifty opaque seeds were picked out of a sample and put through a germination test along with fifty translucent seeds from the same sample. Of the fifty opaque seeds only two grew and of the translucent forty grew. All the seeds were examined microscopically after the test and it was found that of the

forty-eight seeds which failed to grow forty-five were heavily infected, three were slightly infected, while of the two which did grow one was only slightly infected and the other was free from the fungus. Of the translucent seeds which grew thirty-five were not infected, and of those which did not germinate five were found to be infected.

Through the very kind co-operation of Messrs David Bell Ltd., of Leith, further tests have been carried out on these lines. The germination of samples was determined, then the percentage of opaque seeds in these samples and, finally, independent germination tests of opaque and translucent seeds were carried out. All through these tests mycological checks were made to ensure that the opaque seeds selected were infected and the translucent ones healthy, and it was found that a high degree of accuracy was attained. In most cases the germination capacity of the infected seeds was higher than in the original test but the average is still very low, about 15–17 %. In one test the analyst selected what she termed as "very dark" and "dark" seeds; the former had a germination capacity of 8 % and the latter 40 %. This seems to suggest that the degree of infection is indicated by the degree of opacity. The actual figures obtained in one of the tests were as follows: germination of the sample as a whole 63 %; percentage of opaque seeds in the sample 25 %; germination capacity of the opaque seed 17 %; and of the translucent seed 84 %.

The results of the tests show that there is a correlation between the occurrence of *Pullularia* and reduction in germination capacity of the seed, but it has not yet been proved to be the only cause of the death of the seed. The fungus has now been found in Irish, Ayrshire and Aberdeenshire seed.

One interesting sample of Danish seed was examined. This seed appeared dark in colour and contained many opaque kernels, but when these were tested 93 % grew. However, it was found on examining the seed microscopically, that *Pullularia* was not present and it was concluded that the dark colour was due to some other factor such as weathering. Experiments have also been carried out on the effect of seed dressings. Treatment had no effect on either opaque or translucent seed. On making sections of infected seeds, hyphae were found in a layer running round the periphery of the kernel in the region of the aleurone layer and penetrating the endosperm. In some seeds the scutellum was transformed into a mass of hyphae rather like a sclerotium, while hyphae seemed also to be present in the tissues of the embryo. At several points hyphae had emerged on the surface of the kernel and formed fructifications containing spores.

Some difficulty has been encountered in differentiating the hyphae of *Pullularia* from that of the endophytic fungus described by Miss McLennan as occurring constantly in the seed of *Lolium perenne*. Sections of translucent seeds showed no hyphae so that it seems reasonable to suppose that the hyphae found in the opaque seeds belonged to *Pullularia*.* However, McLennan records the following observation: "When examining a sample of English rye grass from Ireland a specimen was occasionally found showing hyphae invading the starchy endosperm. A careful examination of the aleurone layer of such a grain showed that the hyphae were also running riot here. In addition the scutellum showed an extraordinarily large amount of the fungus. These phenomena were not confined to the sample from Ireland; one of English rye grass from South Africa also contained certain grains showing an extraordinary distribution and growth of the fungus. As before, both the aleurone layer and the scutellum were

* The sections referred to were cut on the freezing microtome as circumstances did not permit the employment of more definitive technique.

permeated by intracellular hyphae. In one particular case the scutellum, which normally is packed with aleurone grains, appeared to consist of a dense sclerotial-like mass of threads."

Although Miss McLennan does not say so, it seems likely that seeds such as these would not grow. Miss Sampson, working with *Epichloë typhina* on *Festuca*, records instances of the endophyte running riot, such seeds being moribund. A few experiments have been started in an attempt to determine the pathogenicity of *Pullularia* towards *Lolium* spp., but results are not yet available.

In conclusion, mention should be made of the various methods employed by seed-testing stations and their effect on fungi, pathogenic or otherwise, which may be present on the seed.

For example, in the testing of the germinating capacity of cereal seeds, in certain stations it is the practice to plant these vertically with the germ downward in trays of sterilized soil, and projecting about half their length from the soil. If the infection of these seeds with species of *Helminthosporium* or *Fusarium* be considered, it is apparent that placing the seeds in this way tends to suppress the symptoms of infection by these fungi, particularly the twisting and distortion which occurs when such infected seedlings force their way through soil and which give rise to the condition known as pre-emergence blight. The test mentioned is not intended primarily as a test of the sanitary condition of the seed, but it seems that the percentage of abnormal seedlings is reduced owing to the artificial method of planting the seed.

This is a case where the development of the fungal flora is suppressed but, in other instances, the saprophytic fungal flora is artificially accentuated. It is sometimes the practice to germinate seeds of Burnet and Sainfoin between sheets of absorbent paper which, in turn, lie on flannel pads. It was found that such seeds were often smothered by saprophytic fungi such as *Mucor* spp. which did not develop if the seeds were planted in soil. A simple experiment was carried out with these seeds and it was found that seeds dressed with a mild fungicide and then germinated in the pads had the same germination percentage as untreated seeds germinated in soil, and that this figure was higher than that of the untreated seeds germinated in the pads. There is provision for treatment of seeds in the International Rules for Seed Testing where, under the heading of Chemicals, it is stated: "Treatment of the seed with chemical solutions to hasten the germination is not allowed. On the other hand, the treatment of seeds with disinfectants such as are in general agricultural practice to eliminate disease, is permissible", provided that this is mentioned on the International Analysis Report and the germination figure of the untreated seed is shown. Probably this rule was really framed to cover such as the dressing of cereals infected with *Helminthosporium*, but it would be interesting to know whether the elimination of saprophytic fungi could legitimately be included.

Also there are such diseases as those caused by *Ascochyta* and *Mycosphaerella*. Infected seeds, in the dry state, do not always show external symptoms which can be recognized but if the seeds are germinated between layers of blotting paper the fungus emerges and forms pycnidia on the surface of the seed, and in this way the infection is easily recognized. If, however, the seeds are germinated in soil the pycnidia do not form so readily and, even when they do occur, it is much more difficult to distinguish them among the soil clinging to the seedling. On the other hand, if the seedlings are allowed to remain longer in soil than is required for the ordinary germination test, infection could later be recognized by the lesions which would then form.

IV. HYBRIDIZATION IN BRASSICAE AND THE OCCASIONAL CONTAMINATION OF SEED STOCKS

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Cross-fertilizing crop plants require isolation not only to separate their varieties, but also to exclude any other forms which may be sufficiently closely related to hybridize with them. Among the *Brassica* crops grown in Britain, the two mustards, *Brassica nigra* ($N=8$ chromosomes) and *Sinapis alba* ($N=12$), are said to be incompatible with one another and with all other forms. The cabbage tribe *Brassica oleracea* ($N=9$), comprises a wild species and cultivated forms such as cabbage, savoy, brussels-sprouts, kohl-rabi, curly kale, thousand-headed kale and marrow-stem kale. These form all intercross more or less readily and have fertile offspring. A third group within which intercrossing is also possible includes turnips and turnip-like rapes, *B. rapa* ($N=10$) and swedes, swede-like rapes and certain kales, *B. Napus* ($N=19$). Until recently it was doubted whether crossing was possible between *B. oleracea* × *B. Rapa* or *B. Napus*, but Roemer (1935) has succeeded in crossing the swede ♀ with several forms of the cabbage tribe. The difficulties of obtaining the cross artificially suggest that there is very little risk of its occurrence in nature. According to U (1935), *B. Napus* is a natural amphidiploid containing genomes of the types constituting *B. oleracea* and *B. Rapa* respectively.

B. Rapa includes white and yellow turnips and two rape forms. These are all interfertile and give fertile hybrids. The turnip-like rapes are not usually grown in Britain, so there is seldom any risk of contamination by these. One is an annual which is grown for oil seed on the Continent, while the other is a biennial, the seed of which is imported under the name of Rübsen or bird seed rape. On occasion this seed has been accidentally sown; it is larger than turnip seed but light red in colour. The plant is worthless as fodder, having turnip-like leaves and a woody root shaped like a malformed sugar beet. The cultivated forms, which have been listed here as *B. Rapa*, are supposed to have been derived from the wild species, *B. campestris*, with which they are compatible. This species is stated by Bentham & Hooker to be a frequent weed of cultivation in Britain. There are also records of colonies which are supposed to have been "escaped" rape forms.

When *B. Rapa* is crossed with *B. Napus* vigorous hybrids are obtained. If one of the parent forms is rape-like, the "bulb" of the hybrid is worthless; but true swede × turnip gives a hybrid which has a very large bulb. This is usually disfigured with "hybrid nodules" and swellings which have been attributed variously to *Bacterium tumifaciens* and other causes. The hybrid has an unbalanced constitution and consequently gives little seed, the offspring being almost invariably monstrosities. Some sort of balance can be regained, however, and the author has bred lines by self-fertilization for six generations. These have remained monstrous in form, but a back-cross in which the hybrid was crossed with swede has given a few lines which have become quite self-fertile and are now fairly good, almost swede-like types. Frandsen & Winge (1932) obtained an amphidiploid, or hexaploid race from their hybridizations of swede and turnip. It is unlikely that contamination by turnip would have any

lasting effect on a stock of swede; the low fertility and striking appearance of the hybrid would lead to its rapid elimination.

The cultivated forms known as *Brassica Napus*, include yellow and white swedes, giant and dwarf rapes and certain kales, which are fully interfertile and yield fertile offspring. The greatest risk to swede stocks comes from the rapes which are widely grown and may be encountered as ground-keepers or as seed in badly cleaned bags or machinery. A common rogue of the swede crop is called the "bastard" or "bulbless bolter". It is most readily observed as a dark green plant standing above the general level of the crop, but not all forms bolt. One sample of green-top swede observed by the writer was highly contaminated with a short bulbless form. This could hardly be detected till the crop was lifted, and it was subsequently found to be identical to dwarf rape. A number of these rogues have been collected and self-fertilized at the Scottish Plant Breeding Station. The progenies were grown alongside authentic samples of rape, and segregating generations of swede × rape. Nearly all the rogues bred true and could be matched with either giant or dwarf rape. As the tallest specimens were usually sent in, there was a somewhat greater tendency for bolting than in average giant rape.

A few of the rogues have proved to be "bastards" in that they behaved like hybrids of swede × rape or like descendants of the cross. Rape has two dominant factors each causing the tissues of the root to be white, and when both are absent the tissues are yellow as in the swede. Flower colour is also connected with these factors, giant rape having a bright lemon yellow, and dwarf rape a dull pale yellow colour. In swede and hybrids with yellow tissues the flower is a dull buff colour. Consequently the rapes, the hybrids and most of the derivatives can be detected by flower colour in a multiplication crop of swede. The swede × rape hybrids can be graded up to have bulbs of large size by repeated crossing with swede and by selection. These bulbs are very stringy and would spoil the quality of a crop, but if high dry-matter content were the basis of selection there would be risk of their inclusion. Occasionally cases of mass-contamination occur and may reach the Law Courts. It is then argued on the one side that the seedsman has failed in his duty and on the other that some form of reversion has occurred. Such a phenomenon has not been observed by the writer. It is possible that in such cases there may be environmental bolting of the whole crop together with some degree of contamination by rape to account for the white roots which have usually been described.

The third set of forms which may be included in *B. Napus*, since they appear to have the same chromosomal complement ($N = 19$), is the rape-kale. There are certain varieties on the market called kales which are quite compatible with the swede. Sutton (1907–8) mentions two such forms, asparagus kale and ragged jack kale. The latter is no longer in cultivation and Sutton's present variety is known as Favourite rape-kale. Fill-gap and hungry-gap kales also belong to this group. A case of contamination recently occurred in Aberdeenshire, where strains adapted to local conditions are propagated by farmers. Plots of swede and fillgap kale were seeded quarter of a mile apart, which for some reason was apparently insufficient isolation, for about 10 % of the swede offspring were hybrid in type, having small bulbs with fang-like roots and curled leaves. The combination of bulb formation and curling of the leaves can be obtained from crossings of this nature, but it appears that genetical linkage may cause repulsion of the more pronounced expressions of these characters.

REFERENCES

(1) Fransden, H. N. & Ö. Winge (1932). *Hereditas, Lund*, **16**.
(2) Roemer, W. (1935). *Z. Zücht.*, **20**, 3.
(3) Sutton, A. W. (1907–8). *J. linn. Soc.* (Bot.), **38**.
(4) U, N. (1935). *Jap. J. Bot.* **7**, 3–4.

V. MODERN METHODS OF SEED DISINFECTION

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The subject of seed disinfection and seed protectives provides an interesting example of the vagaries of fashion, and if time had permitted I should have liked to quote one or two of the treatments which have been advocated in the past.

It is, however, modern materials and methods with which I must deal, with the wet treatments such as copper sulphate and formalin, and the dry treatments using basic copper carbonate powders and those proprietary dusts which contain organo-mercury compounds as their fungicidal basis.

Copper sulphate is a good fungicide for the control of covered smut of wheat but it has the drawback that it may decrease germination and this reduction in some circumstances may be high; moreover, if not carefully carried out, abnormal seedlings are frequently produced. It is not a suitable fungicide for oats or barley as the vitality of these grains is injured by such treatment. Formalin is the better fungicide and excellent control of the smut diseases—excluding loose smut of wheat and loose smut of barley—can be obtained; moreover, germination is less impaired.

There are several objections to wet treatments. The moistening of seed grain is a troublesome and laborious operation, and it is necessary to dry the grain quickly after treatment and to sow as soon as possible, otherwise germination may be affected and abnormal seedlings result. It is not surprising, therefore, that dry powders have been developed as seed disinfectants. One of the first employed in this country was a finely divided basic copper carbonate which was used at the rate of 2 oz. per Imperial bushel of grain and from 1923 onwards trials with this dust were made by several workers. For the control of bunt of wheat it was moderately effective provided that the contamination of the grain was not heavy, but its vogue in this country was of short duration although it is interesting to recall that its use in Australia is extensive and most satisfactory. Relatively few farmers used this material and their first introductions to dust disinfectants for seed treatment were proprietary articles containing certain salts of mercury as their fungicidal basis.

Formalin, although an effective fungicide for bunt of wheat, covered smut of barley and smut of oats, did not give a satisfactory control of leaf stripe of barley and leaf spot of oats, and very frequently these diseases were responsible for serious losses and were causing much concern to farmers. In the control of these *Helminthosporium* diseases the organo-mercury seed disinfectants were strikingly successful, and in addition gave good control of the covered smuts. In some cases, however, the control of oat smut was not as effective as a formalin treatment, and it is suggested here that future researches might remedy—or perhaps have already remedied—this defect, for the disease is an important one and more prevalent than is generally realized.

It must be admitted that the introduction of these products is an advance of fundamental importance in the history of synthetic fungicides, as a transition from products of specific fungicidal activity to materials of general fungicidal properties has almost been achieved. The credit for this development must be given to interested commercial firms, for it is very largely by their activities that this result has been obtained.

Among some farmers there is an impression that these dusts are a panacea for all ills to which corn is subject and not infrequently complaints are made that after seed treatment disease has appeared in the crop. Enquiry shows that the diseases in question are loose smut of wheat and leaf blotch of barley. Many observers consider that loose smut of wheat is becoming more prevalent and intense, but it may well be that this apparent increase is connected with the introduction of certain French wheats which appear to be more susceptible to the disease. The grower, not unnaturally, frequently confuses leaf blotch with leaf stripe. Fortunately the former disease cannot be regarded as of serious importance although it probably does affect both the yield and quality of barley, but the seed disinfectant cannot be held at fault as we have no evidence that the disease is seed-borne. Both dusted and undusted seed from a very badly affected barley crop has been sown and perfectly clean seedlings have resulted; indeed, it is not generally until the end of May that we notice the disease at the barley auricles. In the season 1935–6 we tested some experimental dusts and the proprietary organo-mercury products then on the market to see if any control of loose smut in wheat was obtained. As might be expected, no such control resulted; it must, of course, be made quite clear that no manufacturer claims such a control.

It is sometimes argued that the terms seed disinfectant and seed protective are synonymous, but this is not always so. A seed disinfectant such as formalin has an immediate but no lasting value as it cannot confer subsequent protection on the seed. A bunted sample of wheat may be treated in the orthodox way with formalin and if sown immediately a healthy crop will result, but if the sack be roughly handled after treatment or the sample deliberately re-contaminated, and the seed sown, disease will appear in the crop. With a modern product this should not occur as subsequent protection is afforded. A seed disinfectant such as formalin would be of little value in protecting healthy seed from soil organisms, for if conditions for the germination of the seed were adverse then it would be in serious competition with a large and variable soil flora and fauna. In this connexion the treatment of peas may afford an example. In experiments which we have carried out the evidence has indicated that for sowings earlier than March, when encountered, the application of a suitable organo-mercury compound in the form of a dust is likely to result in an increased stand and yield of marketable pods. For sowings later than this there is evidence that the advantages of the application of such a dressing are doubtful.

In what has been said it may have been implied that the only seed protectives are of the organo-mercury type; there are, however, as you are aware, other fungicides such as cuprous oxide which are of value for specific purposes of disease control, or calomel dust which is a protective against specific insect pests. It is, however, with the organo-mercury seed protectives that I shall deal. The composition of these varies with the particular commercial product but the main ingredients of them are very finely divided inert mineral carriers or fillers with which are mixed or impregnated small quantities of one or more organo-mercury compounds—an anti-dust, sometimes

of an oily character, and a dye are often added. In the majority of cases the fungicidal salt concerned would appear to be a member of the series R Hg X, where R is a hydrocarbon and X an acidic radicle. In some cases mixtures of these salts are used. In this series there is a close relationship between composition and fungicidal power, the toxicity decreasing with the increase of the molecule of R. This, however, must not be interpreted as meaning that dusts which may contain phenyl or tolyl compounds are less effective in disease control than those containing ethyl salts. There is evidence that the most fungicidal salts are the methyl compounds but their very poisonous nature precludes (or may preclude) their use as seed disinfectants or protectives.

A modern commercial seed protective, apart from its general or specific fungicidal power and the control which it gives, or should give, of disease arising from the sowing of seed which is contaminated or superficially infected with disease organisms, must be reasonably safe for the operator to use, taking the usual precautions when dealing with a poison: moreover, possible risks of phytocidal injuries must be minimized. Admittedly these newer dust treatments are very effective in disease control but these newer methods are often criticized on the following counts:

(1) The treatment necessitates the purchase of a machine for treating the grain.

(2) It involves recurrent labour charges for de-sacking, dressing and re-sacking the seed, and the cleansing of sacks which have held treated grain.

(3) The disinfectant dusts are poisonous and consequently precautions must be taken when using them.

The first criticism is one which may be discounted, for there are on the market several relatively inexpensive types of machines which serve their purpose well and the upkeep of which is negligible.

The second criticism cannot be considered a major one as the recurrent labour charges are very similar to those involved in seed treatment with the older liquid preparations. Perhaps the most valid argument is that these dusts contain certain compounds which are poisonous and consequently the usual precautions in dealing with a poison should be observed. It must, however, be realized that approximately 97–99 % of the dust which is used consists of the inert filler or carrier and 1–3 % only—and sometimes less—of the poisonous salt.

Surely if elementary precautions are taken the dusting of the grain should be reasonably safe. When, however, this dusting operation is carried out for very extended periods—as it might be by seed merchants—then the greater element of risk must be considered and all adequate precautions taken.

The manufacturers of these products state that it is advisable to avoid inhaling the dust, and that the nose or mouth should be protected with a cloth or respirator. Also, that some products should not be handled with wet or moist hands and should not be allowed to come into contact with wounds or broken skin since they have a vesicatory action. Further, they state that after the treatment has been carried out, any dressing remaining on the hands should be removed by rinsing thoroughly. Also, dressed seed should not be used for feeding purposes, and bags which have contained dressed seed should be shaken out or washed before being used for carrying fodder.

If the machine used for the purpose of dusting the grain is not adequately dust-proof, or if the compound used is volatile, then it is clear that the operator cannot avoid inhaling some of the disinfectant dust and, consequently, if dust-proof conditions cannot be secured, masks or respirators should be worn. Risk would be further

minimized by carrying out the dusting operation in the open air and not in a closed barn or shed. The practice sometimes adopted of spreading the grain on the barn floor, scattering the powder over this and then turning the grain several times with a shovel is to be condemned for two reasons. The ensuing disease control is less effective, and the operator will inhale the dust particles. If any of the above arguments which are sometimes advanced can be considered valid, an obvious corrective can be suggested and that is a combined seed drill and duster, i.e. a machine that would dust the grain as it was being drilled. Some years ago we devised a machine of this type (Leake & Weston, 1938) and it was used on a commercial farm in Cambridge.

Although the trials indicated that the mechanism gave adequate control for practical commercial purposes, it was thought that it would not give that complete control which would be desired by the grower of seed grain for the market. It should, perhaps, be pointed out that the results were obtained with a mechanism designed merely to test a given principle and that this mechanism is capable of modification to give better results. Apart from any question of toxicity a suitable machine of this kind would minimize labour charges.

There can, I think, be little question that the disinfection of seed is now more general than formerly, and we would expect to find that there had been a general reduction in seed-borne diseases throughout the country. It is difficult to obtain any reliable estimates or statistics on this matter but field experience in East Anglia indicates that such diseases as bunt in wheat, leaf spot of oats and stripe of barley have been very considerably reduced, particularly the *Helminthosporium* diseases. As I thought that this question of the prevalence of disease organisms in seed samples was a matter of much interest, I asked the Director of the Official Seed Testing Station, Mr A. Eastham, if he could supply me with any information. As you may be aware, the routine purity examination of cereal samples at this Station includes noting and reporting the presence of seed-borne diseases, where evidence of contamination can be determined by unaided-eye examination only. Based on these examinations, Mr C. C. Brett has very kindly prepared for me figures showing the percentage of bunted wheat samples received each season during the past 20 years. These estimates are shown in Table I.

This low percentage of infected samples during the past three seasons may have some significance, especially in view of the increased number of samples over those years. Such samples, however, represent but a fraction of the total seed stocks which are sown, and most millers are still all too familiar with bunted wheat. As a further

Table I. *Percentage of bunted wheat samples received each season*

Season	1918–19	1919–20	1920–21	1921–22	1922–23	1923–24	1924–25
Percentage of bunt	5·7	6·5	8·0	33·0	15·7	16·0	14·5
No. of wheat samples examined	3729	1048	1926	2312	1866	1830	2326
Season	1925–26	1926–27	1927–28	1928–29	1929–30	1930–31	1931–32
Percentage of bunt	11·9	5·6	7·3	5·7	4·8	4·6	6·2
No. of wheat samples examined	2340	2423	2685	2538	1782	2142	2741
Season	1932–33	1933–34	1934–35	1935–36	1936–37	1937–38	
Percentage of bunt	5·1	3·9	4·1	2·4	2·7	2·0	
No. of wheat samples examined	4575	4465	4896	5991	7499	8101	

bearing it may be of interest to record that for several years past it has become more difficult to obtain contaminated or infected stocks for experimental purposes, especially samples of oats and barley infected with *Helminthosporium*.

Frequently, the question arises as to which is the best seed protective for a farmer to use. The answer must be given that the products on the market are on a competitive basis and that there is no existing organization in this country whereby such materials can be tested officially.

Another question occasionally asked is, if these products can in any way injure the seed—members of the series *R* Hg *X* have a characteristic phytocidal effect when applied in overdoses. The seed commences to germinate and the coleoptile appears, but develops abnormally and swells. Root development is restricted in varying degrees. These "aborted" seedlings seldom develop further and do not rapidly decay. Some instances of this type of injury have been noted in practice but they are rare and the conditions under which it has taken place are obscure.

It has already been mentioned that the organo-mercury protectives give no control of loose smut of wheat and that this disease is very prevalent although not very intense. Have modern researches resulted in any effective and practical treatments? In India, Prof. Jai Chand Luthra (1934) obtained a complete control in many cases and in a very simple way. He pre-soaked samples of smutted wheat in water at ordinary temperature for 4 hr., i.e. 8 a.m. to 12 noon; they were then taken out and exposed to the sun from 12 noon till 4 p.m. The crop raised from the treated seed was quite free from the disease, while the control plots of untreated seed had as much as 14 % of smutted heads. It is clear, I think, that such a treatment would have no success here.

In East Anglia we do not advise farmers to carry out the hot-water treatment for the control of loose smut of wheat as the risk of destroying the vitality of the grain is so great, but it is interesting to record that some seed merchants are now experimenting with hot-water treatments.

I have dealt almost entirely with the cereal diseases, but the beneficial vogue of seed treatment is not confined to these crops alone, for by these or other methods a wide range of seeds are now often treated prior to sowing. But is this a modern conception? Peters (1771) in *Winter Riches* says: "Shall I stand alone convicted of all mankind, stand a culprit before my great superiors, when I affirm the great omission in not reminding the farmer, that it is absolutely as necessary for him to take the same precautions, care, and assiduity through every species of grain he commits to the benevolent earth, as to wheat," and concluded, "Is it because they are not of equal value—too vague a word to answer? Let me therefore recommend that duty (you owe it to yourselves) as much to one, as the other; they are all fed with the same food, and nourished in the same earthly bowels...."

REFERENCES

Leake, H. M. & Weston, W. A. R. Dillon (1938). Combined seed drill and duster. *J. Minist. Agric.* **45**, 344.

Luthra, Jai Chand (1934). Some experiments on the control of loose smut, *Ustilago Tritici* (Pers.) Jens. of wheat. *Indian J. agric. Sci.* **4** (1).

—— (1934). The loose smut disease of wheat (vernacular "Kangiari") and some new methods of its control. *Agric. Live-Stk, India*, **4** (5).

Peters, M. (1771). *Winter Riches*, p. 137.

REVIEWS

Practical Lawn Craft. By R. B. Dawson. Foreword by R. G. Stapledon. Pp. 300+32 illustrations and 3 text-figures. London: Crosby Lockwood and Son, Ltd. 1939. 15*s.* 0*d.*

The author of this volume is the Director to the Board of Greenkeeping Research, St Ives Research Station, Bingley, Yorkshire. He has held this post since the establishment of the Station in 1929, and in his capacity as Director, has carried out a vast number of experiments on all kinds of turf problems and has had ample opportunity of examining sports turf in all parts of Great Britain and many parts of the Continent. This book embodies the results of these experiments, and includes also an account of the work of other investigators of turf problems.

It is divided into five sections dealing respectively with the historical aspect of lawns, the formation of new lawns, the maintenance of established turf, turf for sport and other purposes, and turf upkeep in other countries. Detailed instructions are given for the construction of new lawns from sods and from seeds mixtures, and the author emphasizes the superiority of simple seeds mixtures, pointing out that "on swards consisting of the finest turf, bent, or a perfect blend of bent and fescue, may account for almost 100 % of the herbage". The maintenance of established turf is treated from every angle, including the choice of a mower, the practical use of fertilizers, eradication of weeds and earthworms, and the various mechanical operations necessary for turf upkeep. Four chapters are devoted to instructions for the management and upkeep of turf for all kinds of sport from bowls to polo, and a whole chapter is concerned with the establishment and upkeep of aerodromes. The whole subject of lawn craft is treated from the scientific point of view, but in such a manner that it can readily be understood and made use of by the practical man. Details of the cost of the various operations necessary for turf upkeep are included, and instructions for mixing fertilizers and for laying out sports grounds of all kinds are given in the appendix.

This book is indispensable for all professional greenkeepers and groundsmen, and for anyone who desires to be the owner of a good lawn. ADELA G. ERITH.

An Introduction to Industrial Mycology. By G. Smith. Pp. xii+302. London: Edward Arnold and Co., Ltd. 1938. 16*s.* 0*d.*

This is not a textbook of fungi: it deals with a very heterogeneous series of common moulds and is meant primarily for industrial chemists lacking mycological training. Industrial microbiologists possess, in nearly all cases, a chemical background rather than a botanical one, but there should be ample scope in this field for botanists specializing in microbiology, although they would need to acquire also a good knowledge of biochemistry, and such students will find this book very useful.

Ch. I is introductory, Ch. II deals briefly with terminology and classification, Ch. III is devoted to the Zygomycetes, Ch. IV to the Ascomycetes, Chs. V and VI to the Fungi Imperfecti, Ch. VII to *Aspergillus* and Ch. VIII to *Penicillium* and related species. Later chapters deal respectively with laboratory equipment and technique, the physiology of mould fungi, the maintenance of a culture collection, the control of mould growth, and the industrial uses of fungi. The final chapter is a brief annotated guide to mycological literature, and the book closes with a good index. There is a commendatory foreword by Prof. H. Raistrick, each chapter terminates with a useful selected bibliography, and several chapters contain dichotomous keys. The selection of material is artificial, being determined by the prevalence and importance of particular genera and species in industry either as contaminations or in relation to various processes. The author's treatment is conditioned by the same factors, the major emphasis being laid upon identification and practical considerations. As these aspects of fungal study are largely or entirely neglected in textbooks of fungi this book forms valuable accessory reading.

The work is extremely well done, the chapters dealing with *Penicillium* and *Aspergillus* being particularly useful. The chapter on laboratory equipment and technique is excellent but note might have been made of the cheapness and usefulness of plain medical flats for large size and stock cultures, and also of the manifold uses of cellophane in culture work. The chapters on the physiology of mould fungi and the industrial uses of fungi might, with advantage, have been made much fuller, and in that on the control of mould growth attention might have been drawn to the use of nipagin. There are occasional misprints and, here and there, sentences requiring modification, e.g. p. 62, "Conidial forms known as *Botrytis* are known to belong to several different genera of Ascomycetes"; p. 228, "Many workers prefer to make cultures which are obtained by germination of a single spore, this being an absolute guarantee of purity".

A notable feature of the book are the 127 beautiful photomicrographs arranged on 64 plates and containing some of the best illustrations of micro-fungi yet published. Although only an introduction to the subject, the book is a first class piece of work and bears the hallmark of research experience. Not only industrial chemists but microbiologists, plant pathologists, and botanists will find it of value. W. B. BRIERLEY.

Cattle Fodder and Human Nutrition: with special reference to Biological Nitrogen Fixation. By ARTTURI I. VIRTANEN. Pp. 108. Cambridge: University Press. 1938. 7*s.* 6*d.*

In 1937 the author, who is the Director of the Biochemical Institute, Helsinki, gave three lectures at London University, and one at Reading University and these, together with a brief survey co-ordinating the material, are here reprinted. Prof. Virtanen's researches have, in recent years, fundamentally changed our conception of the nitrogen fixation process by root nodule bacteria, and of the way in which the nitrogen becomes utilizable by leguminous and other plants, although certain of his results have not obtained confirmation on repetition of the experiments by workers in other countries. The first two lectures deal with this subject and are entitled "The

Mechanism of the Biological N-Fixation" and "The Symbiosis of the Leguminous Plants with the Legume Bacteria".

The last two lectures deal with "The Production of Vitamins in Agriculture, with Special Reference to Human Nutrition", and "The A.I.V. Method for the Preservation of Fresh Fodder and its Importance in Agriculture". At first glance these subjects seem to bear little relation to those dealt with in Lectures I and II, but the researches on which they are based arose directly out of the author's investigations on leguminous plants. Lecture III has particular reference to conditions in Finland where winter fruits and vegetables are not easy to obtain, but has also considerably wider application. The lack of vitamin A in the winter rations of cattle in Finland led Prof. Virtanen to seek a method whereby fresh, young, digestible and protein-rich fodder could be made available for this purpose, and this eventuated in his well known A.I.V. silage process, the principle of which is that the *p*H of the fodder mass must be below *p*H 4 in order to guarantee a successful conservation. The adoption throughout Finland of the A.I.V. method has solved the vitamin problem in that country, but the process is also of the utmost value in many other regions. Prof. Virtanen is of opinion "that the A.I.V. method will replace, to a great extent, the earlier method of haymaking" and that "the artificial drying of grass, by means of which the same results are obtained as by the A.I.V. process, has no economic possibilities, at least in its present form, of gaining ground in the preservation of fodder".

The author's concluding "General Survey" occupies only a little over three pages, but it epitomizes in a remarkable way the matter and viewpoints elaborated in the previous 100 pages, and it is of first class interest. As much of Prof. Virtanen's original work is published in rather inaccessible Continental journals it is very useful to have this authoritative and up-to-date account in so convenient a form.

W. B. BRIERLEY.

Coffee in Kenya. By the Staff of the Scott Agricultural Laboratories and the Agricultural Economist, Department of Agriculture, Kenya, with a contribution by the Director of the British East African Meteorological Service. Edited by J. McDONALD. Pp. vi+210. Plates 26. Nairobi: Government Printer. 1938. 5*s.* 0*d.*

In his foreword, Mr H. B. Waters, Director of Agriculture in Kenya, states that "This publication is not to be regarded as a textbook on coffee which will serve to teach planters the A.B.C. of coffee cultivation, but as offering to planters some elucidation of problems met with in Kenya plantations". It contains six parts as follows: I, The climate of the coffee areas of Kenya, by A. Walter (pp. 18); II, Coffee soils and their treatment, by G. H. Gethin Jones (pp. 37); III, Cultural practice and factory treatment, by A. D. le Poer Trench, S. Gillett, and T. L. McClelland (pp. 54); IV, Insect pests of coffee, by F. B. Notley (pp. 36); V, Diseases of coffee, by J. McDonald (pp. 43); VI, Economics, by V. Liversage (pp. 13); The book contains numerous diagrams and tables and concludes with an index. The text is clearly and interestingly written although, in the foreword, there is a curious mistake in the dating of the Great War and in Part III the author uses the rather obsolete adjective "fungoid".

The book should serve its purpose extremely well and, although it relates specifically to Kenya, it should have a wider appeal since it has something of the nature of a preliminary monograph on the bionomics of *Coffea arabica*, and it is a useful supplement to its editor's *Coffee Growing, with Special Reference to East Africa*, published in 1930. Research on coffee in Kenya only began about 1924–6 and this publication is a most creditable record of what has been achieved in the intervening period.

W. B. BRIERLEY.

Third Conference on Cotton Growing Problems: Report and Summary of Proceedings. Pp. 168. Empire Cotton Growing Corporation, King's Buildings, Dean Stanley St., London, S.W. 1. 1938. 2*s.* 6*d.*

The Conference was held in London on 22 and 23 September 1938, and was attended by scientific workers representing all cotton growing regions of the Empire. The Report contains a summarized introduction of each paper by the author together with the discussion thereon, and the contributions are grouped under the following heads: A, The place of cotton in agricultural systems; B, Local problems of cotton growing; C, The manuring and cultivation of cotton; D, Some problems other than manuring; E, Diseases and pests of cotton, (1) general, (2) food supply as a factor in insect attack outbreaks, (3) dormancy in insects and its relation to survival; F, Fibre properties of cotton. Two more general papers which do not fit into the above scheme are intercalated, viz. "Systematic observations and studies of plant development in field plots of cotton, and in replicated field experiments on cultivations, manuring and other matters", and "Some problems in genetics whose solution would help the plant breeders".

The width of experience represented in the contributions makes them especially interesting, and the Report is a valuable summary of present knowledge of the particular problems at issue.

W. B. BRIERLEY.

Plant Injection for Diagnostic and Curative Purposes. By W. A. Roach. Technical Communication No. 10. Pp. 78. East Malling: Imperial Bureau of Horticulture and Plantation Crops. 1938. 5*s.* 0*d.*

Dr Roach has made this subject peculiarly his own and in this Bulletin gives a clear account of his ingenious and fruitful researches. Following an interesting introduction the author discusses fully and critically the methods of plant injection. This section, which forms the bulk of the work, is a masterly piece of writing and is beautifully illustrated. It is followed by brief sections concerned with the determination of the distribution of injected liquids, damage to foliage resulting from injection, dosage, localization of effects of injection, injection of plants when leafless, solid injection, and an all too brief consideration of the general application of plant injection methods. The Bulletin concludes with a short general summary and a useful bibliography of 162 citations.

For a long time botanists have, in a desultory sort of way, been interested in plant injection since on *a priori* grounds it ought to be a valuable and illuminating method of research. On the other hand, the use made of it and the results accruing have never seemed to fulfil its inherent promise. Much of the importance of Dr Roach's work, as Prof. V. H. Blackman points out in his foreword, resides in the fact that he has developed plant injection "into a precise technique which can be followed by others; he has thus placed in our hands a new instrument of great value in both pure and applied biology".

W. B. BRIERLEY.

Plant Physiology. By E. C. Miller. 2nd edition. Pp. xxxi+1201. London: McGraw-Hill Publishing Co., Ltd. 1939. 45*s.* 0*d.*

In the new edition of this well-known treatise the general framework and chapter headings remain unaltered, but the treatment of various topics has been revised or amplified, and certain new topics have been added. Even with extensive use of small type the inclusion of the new material has necessitated an increase of 300 pages in the size of the volume. There are two new text-figures, the questions concluding each chapter in the first edition have advisably been omitted, and the number of bibliographic citations has been more than doubled. The author's general aims and points of view have not changed, his consideration is strictly physiological in so far as physiology can be separated from biochemistry, and as befits a book by a Professor in an agricultural institution the work has a slight but welcome agricultural bouquet. As in the first edition the text is restricted to the physiology of the green plant and such subjects as microbial physiology, or pathological physiology, except in so far as other nutritional troubles are concerned, are excluded.

During the period elapsing between the editions advance has been made in all directions but more especially, perhaps, in knowledge of the plant cell, transpiration relationships, carbohydrate formation, nitrogen metabolism, translocation, and respiration, and notably in the extension of our knowledge concerning the elements absorbed by plants, and in our understanding of the process of growth in plants. For example, in relation to growth processes, the whole subject of phasic development with its far-reaching theoretical implications, and its practical significance in the vernalization of crops, is essentially a development of this decade. Also, although the existence of growth-promoting and growth-inhibiting substances has been known for some 20 years, all our understanding of them and of their fundamental importance has come during the last 10 years. Similarly, during this period there has been a great extension of our knowledge concerning the role of the minor elements in plant nutrition. All these advances have been incorporated very skilfully by Prof. Miller in his book which is densely packed with information, but since the new edition has developed from the old by transformation rather than by apposition or intussusception, and because the more technical and detailed matter is relegated to small type, the book still retains its readable quality. Some of the new developments are not so straightforward as the older classical material and are, on occasion, subject to controversy, but the author has succeeded in being impartial in his presentation of data and viewpoints.

During the last 10–15 years numerous plant physiological works in English have been published. When the first edition of this book appeared in 1931 it was at once recognized as a contribution of outstanding merit, and it has successfully stood the test of time and usage. The second edition is even better than the first, and as a source-book of information and a guide to the literature of the physiology of the green plant it has no compeer.

W. B. BRIERLEY.

Principles of Genetics. By E. W. Sinnott and L. C. Dunn. 3rd edition. Pp. xiv + 408. London: McGraw-Hill Publishing Co., Ltd. 1939. 21*s.* 0*d.*

The two authors are Columbia University Professors of Botany and Zoology respectively, and they form an ideal combination. Their book has long been recognized as one of the best general introductions to the subject, and the third edition will maintain this reputation. The new data and viewpoints developed since 1932 have been incorporated and, although modern genetics is far from being an easy subject, the authors have succeeded in giving a balanced, accurate, and up-to-date picture of the situation in readable and interesting language. The new data largely concern gene locations and chromosome changes, but the discussions of cytoplasmic inheritance, heterosis, multiple factor inheritance, mutation frequency, and polyploidy have been amplified. By condensation of matter, and redistribution of the "Problems" and the statistical material, the new edition has been shortened by 33 pages. Many of the illustrations of the earlier book have been omitted or replaced by better figures, and the chapter bibliographies have been brought up to date.

Any student working through this book and tackling the "Problems" seriously will acquire a very sound introductory knowledge of genetics.

W. B. BRIERLEY.

The Genetics of Garden Plants. By M. B. Crane and W. J. C. Lawrence. 2nd edition. Pp. 21 + 287, figs. 62 and 43 tables. London: Macmillan and Co., Ltd. 1938. 12*s.* 6*d.*

The first edition of this work appeared in 1934 and received notice in the *Annals* (1935), **22**, 437. The general plan of the book and the mode of presenting material remain the same, but in every chapter there have been considerable re-writing and addition of new data and points of view to bring the work up to date. The main structural change is the addition of a new and valuable chapter on the chemical and genetical basis of flower colour, in which is incorporated an account of *Dahlia variabilis* which, in the first edition, formed part of the chapter dealing with flowering and ornamental plants. This new chapter is, perhaps, the best simple account of this difficult subject available. Also, in the new edition there is a useful appendix listing the chromosome numbers of plants mentioned in the text, and the bibliography has been considerably extended.

A reader familiar with the groundwork of genetics will find the first three chapters an excellent summary of genetical principles, and the remaining chapters a first class account of their exemplification in horticultural plants.

W. B. BRIERLEY.

The Cultivation of Succulents. By H. JACOBSEN. Authorized translation by VERA HIGGINS. Pp. 106. 8 figs. London: Williams and Norgate, Ltd. 1939. 5*s.* net.

Anyone interested in the succulent plants is familiar with Jacobsen's monograph *Succulent Plants.* In this botanical details of the species were given but only occasional notes on the cultivation of those requiring special treatment. The present volume is to be regarded as supplementary to that work and gives full details of the methods of propagation, the uses and the cultivation in pots and in the open of this type of plant. Interesting chapters are also included on the distribution in nature of succulents and the methods of collection and importation. The translator, herself a well-known authority on the subject and author of several books on cacti, is to be congratulated on making available to the increasing number of enthusiasts in this country a book which contains so much detailed information in so small a compass. R. H. STOUGHTON.

Research and Statistical Methodology: Books and Reviews, 1933–8. Edited by O. K. BUROS. Pp. vi+100. New Brunswick: Rutgers University Press. 1938. $1.25.

Dr Buros has collected and classified the titles of "practically all the research and statistical methodology books published between 1 January 1933 and 15 November 1938 and written in the English language. For each book, full bibliographic information is given including both American and English prices and publishers. Hundreds of journals in numerous fields were searched for reviews of these books. The most critical portions of the reviews were then excerpted and collated as published herein." For example, B. 700 is a citation of Snedecor's *Statistical Methods* with details of publisher, price, etc., followed by excerpts from reviews published in ten scientific journals, in each case citing the issue of the journal, and naming the reviewer.

It is an unusual sort of publication but decidedly useful to librarians and others desiring to know scientific opinion on books before purchasing them; continued as periodic issues the series will be of real value as a source of reference.

W. B. BRIERLEY.

Methods and Material for Teaching Biological Sciences. By D. F. MILLER and G. W. BLAYDES. Pp. xii+435. London: McGraw-Hill Publishing Co., Ltd. 1938. 21*s.* 0*d.*

This book is intended for teachers of elementary biology and for teachers in training. Part I, "Principles and Classroom Methods", is designed primarily as a text for classes in special methods and deals with the biological basis of education, the objectives of teaching in biological subjects, types of courses, methods of presentation, making a teaching plan, an evaluation programme, the lack of materials and equipment, visual education, how to choose a text, and trends in the curriculum. Part II,

"Preparation and Uses of Classroom Materials", deals more practically with examples of student projects, collecting, culturing and preserving of material, laboratory aids and substitutes, microscopical preparations, and methods and materials suitable for classroom demonstrations and experiments dealing with photosynthesis, digestion, nutrition, and growth, diffusion, circulation, respiration, water relations of plants, the response of organisms, reproduction, and heredity. The book is illustrated by 146 text-figures, contains numerous test questions and useful bibliographies, and concludes with an index.

The book has particular reference to American conditions and problems but much of it is of quite general application. Part I is interesting and suggestive, although here and there a little trite. Part II, occupying two-thirds of the volume, will be useful to all teachers of elementary biology since it is packed with data and practical suggestions concerning the materials and methods of laboratory preparation and teaching.

W. B. BRIERLEY.